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Analytical Control Test Plan and Microbiological Methods for the Water Recovery Test

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ACRONYMS

"A" Data	Acceptable Data
AAM	American Academy of Microbiology
ACC	Analytical Control Coordinator
ACS	American Chemical Society
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ASCP	American Society of Clinical Pathologists
ASTM	American Society for Testing and Materials
BD & SC	Boeing Environmental Laboratory
BNA	Base Neutral and Acid Extractables
BOD	Biological Oxygen Demand
CAL	Calibration Standard
COD	Chemical Oxygen Demand
EDTA	Disodium Salt of Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
FD 1 & 2	Field Duplicate 1 & 2
FEDS	Freedom Environmental Database System
FRB	Field Reagent Blank
JSC	Johnson Space Flight Center
LD 1 & 2	Laboratory Duplicate 1 & 2
LFB	Laboratory Fortified Blank
LFM	Laboratory Fortified Sample Matrix
LPC	Laboratory Performance Check Solution
LRB	Laboratory Reagent Blank
MBAS	Methylene Blue Activated Substances
MSFC	Marshall Space Flight Center
"N" Data	Not Controlled Data
NASA	National Aeronautics and Space Administration
NIOSH	National Institute for Occupational Safety and Health
NVO	Non-Volatile Organic Compound
QA/QC	Quality Assurance/Quality Control
QCS	Quality Control Standard
"S" Data	Suspect Data
SVOC	Semi-Volatile Organic Compound
TBD	To Be Determined
TDS	Total Dissolved Solids
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solids
USEPA	(See EPA)
VOA	Volatile Organic Acid
VOC	Volatile Organic Compound

ANALYTICAL CONTROL TEST PLAN AND MICROBIOLOGICAL METHODS FOR THE WATER RECOVERY SYSTEMS TESTS

VERSION 3.3

1.0 INTRODUCTION

Qualitative and quantative laboratory results are very important to the decision-making process. In some cases, they may represent the only basis for deciding between two or more given options or processes. Therefore, it is essential that handling of laboratory samples and analytical operations employed are performed at a deliberate level of conscientious effort. Reporting erroneous results can lead to faulty interpretations and result in misinformed decisions[1].

1.1 Scope

This document provides analytical control specifications which will govern future test procedures related to all WRT Phase III activities to be conducted at the National Aeronautics and Space Administration/Marshall Space Flight Center (NASA/MSFC). This document addresses the process which will be used to verify analytical data generated throughout the test period, and to identify responsibilities of key personnel and participating laboratories, the chains of communication to be followed **(see Appendix 9)**, and ensure that approved methodology and procedures are used during Water Recovery Test (WRT) activities. This document does not outline specifics, but provides a minimum guideline by which sampling protocols, analysis methodologies, test site operations, and laboratory operations should be developed.

This is Version 3.3 of the Analytical Control Test Plan. Versions 1.0-3.2 were working copies, and have not been formally published by NASA, or any NASA organization or contractor. Version 3.3 represents the most up-to-date information available at the date of printing, and has been developed in a generic format excluding specific names and organizations.

1.2 Analytical Control Coordinator

An Analytical Control Coordinator (ACC), was appointed by NASA/MSFC, ED62, to implement and monitor the program described within this document. The ACC is available to respond to questions and concerns and will be required to make periodic unannounced inspections of sampling and shipping procedures during NASA WRT activities **(see Appendix 1)**. The ACC is responsible for monitoring sample collection activities **(Appendix 1)** and preparing blind duplicate and blind reference samples **(Appendix 2 and Section 3.3.1)**. In addition, the ACC is responsible for implementing and monitoring the interlaboratory verification program and other Quality Assurance/Quality Control (QA/QC) activities **(Section 3.3)**. The ACC is, in addition, responsible for the revision of the AC Plan to meet the requirements of specific tests, and the development of NASA approved Standard Operating Procedures (SOPs) for all ACC regulated activities.

All ACC concerns regarding the WRT will be reported to NASA ED62. The ACC will be available for meetings at NASA, both scheduled and impromptu. These meetings will be held primarily at ED62, Building 4610, MSFC, and Building 4755, MSFC (the WRT test site). Other meetings to be attended by the ACC may be held at MSFC Headquarters (Building 4200), and NASA prime contractor facilities. The ACC will possess a minimum of a Bachelor of Science degree, five years experience in QA/QC and analytical chemistry, and good oral and written communication skills.

1.3 Summary

The interlaboratory control verification program consists of periodic evaluations of laboratory performance and monitoring the quality of the analytical results. Evaluation of laboratory performance is conducted via the qualification and verification programs described in **Section 3.3**. The determination of the quality of analytical results is accomplished using blind reference samples. The results submitted for the blind control samples (included with all appropriate batches) will be reviewed by the ACC and the corresponding parameters within that sample batch will be identified as "acceptable" or "suspect" based on these results **(see Appendices 3 & 7)**. It should be understood that "Suspect" data does not place blame with any particular group. When data is marked "Suspect", this only indicates that the QC associated with the parameter has identified a problem. The problem may lie with the participating laboratory, the ACC, and/or the control sample itself. For this reason, "Suspect" data should be treated with scrutiny but blame for the problem should not be directed to **any one** individual or organization. By treating data in this way, all parties involved in WRT work will be ensured that the data generated is of the highest possible quality. Ratings (A or S) will be assigned by the ECLSS database, the Freedom Environmental Database System (FEDS), based on reference information loaded by the ACC. The Data Custodian will report QA/QC data directly to the Principal Investigator and Test Conductor. The Data Custodian is responsible for the logging and transmission of all data pertaining to the WRT, to those parties in need of the data. The action will be carried out by providing daily reports generated by the FEDS. All data relating to test subject safeguards will additionally be released directly to the Medical Monitor. Additional information regarding the transfer of information from the Data Custodian may be found in **Appendix 9**.

Please review the SOPs, located in the Appendices of this document, for detailed procedures utilized in the WRT. Any questions pertaining to this document, including the SOPs, should be directed to the ACC through NASA/MSFC, ED62.

2.0 APPLICABLE DOCUMENTS

The following documents were used in the development of this AC Plan. In addition, several of these documents are used as reference guides (by the ACC and others) during WRT activities.

1. "Annual Book of ASTM Standards," Section 11, Water and Environmental Technology," American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103, 1987.

2. "Annual Book of ASTM Standards, Part 31," Water, American Society for Testing and Materials, Philadelphia, PA 19103, 1981.

3. Eichelberger, J. W. and W. L. Budde, "Method 524.1, Measurement of Purgeable Organic Compounds in Water by Packed Column Gas Chromatography/Mass Spectrometry," Revision 3.0, Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio, 45268, 1989.

4. "EPA Good Laboratory Practice Compliance Inspection Manual," Government Institutes, Inc., 966 Hungerford Drive, Rockville, MD 20850, 1987.

5. Garfield, Frederick M., "Quality Assurance Principles for Analytical Laboratories," Association of Official Analytical Chemists, 1111 North 19th Street, Suite 210, Arlington, VA 22209, 1987.

6. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA 600/4-79-019, USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, 1979.

7. Kateman, Gerrit, "Quality Control in Analytical Chemistry," John Wiley and Sons, 605 Third Avenue, New York, NY 10158, 1981.

8. "Manual of Methods for Chemical Analysis of Water and Wastes," EPA 600/4-79-022, USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45263, 1983.

9. "Microbiological Methods for Monitoring the Environment, Waters and Wastes," EPA 600/8-78-017, USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, 1978.

10. "Methods of Air Sampling and Analysis," M. Katz, ed., 2nd ed., American Public Health Association, Washington, D.C., 1977.

11. Miller, J. M. and Wentworth, B. B. eds., "Methods for Quality Control in Diagnostic Microbiology," American Public Health Association, Washington, DC, 1985.

12. "NIOSH Manual of Analytical Methods," 3rd edition, National Institute for Occupational Safety and Health, Cincinnati, OH 45226, 1987.

13. "NIOSH Specifications for Industrial Hygiene Laboratory Quality Program Requirements," National Institute for Occupational Safety and Health, Cincinnati, OH, 45226. 1976.

14. Riggin, R. M. "Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air," EPA-600/4-84-041, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, 1984.

15. Sherma, J. ed., "Manual of Analytical Quality Control for Pesticides in Human and Environmental Media," EPA, 600/1-79-008, USEPA, Research Triangle Park, NC, 27711, 1976.

16. "Standard Methods for the Examination of Water and Wastewater," 16th edition, American Public Health Association, 1015 18th Street NW, Washington, DC 20036, 1985.

17. "Standard Methods for the Examination of Water and Wastewater," 17th edition, American Public Health Association, 1015 18th Street NW, Washington, DC 20036, 1989.

3.0 REQUIREMENTS FOR THE CHEMICAL ANALYSIS OF WATER

3.1 Sample Collection and Control Procedures

The control of analytical performance begins prior to the actual collection of samples. All control procedures for sample tracking including sample collection, preservation, analysis, storage, and disposal should be in compliance with approved U.S. Environmental Protection Agency (EPA), American Society for Testing and Materials (ASTM), American Public Health Association (APHA), and/or NASA/ACC reviewed and approved procedures. For specific or more detailed information, consult the applicable documents listed in **Section 2.0** of this document. All unusual sample collection techniques required must be reviewed by the MSFC Material and Processing Laboratory and the Principal Investigator prior to ACC approval.

3.1.1 Sample Containers

Containers for the collection of various samples will meet or exceed all APHA, ASTM, and/or EPA requirements[2-4].

3.1.2 Sample Collection

Sample collection should be accomplished by procedures described by the APHA and/or EPA unless specified otherwise and authorized by NASA[2,3]. All samples will be collected by technicians trained in aseptic techniques. A minimum number of persons should be involved in the actual sampling process. Written procedures must be available on-site for reference and review by sampling personnel. These procedures will be made available to the samplers by their employer. Prior to collection of the actual sample(s), from a specific location, at least one hundred milliliters of water will be voided through the collection port. If microbiological samples are to be collected, the sample port will be adequately disinfected prior to sample collection. If both chemical and microbiological samples are to be collected from a given sample location, the chemical sample is collected first immediately following initial flushing. Once all the samples are collected, for the chemical parameters, the port is disinfected (heated with a high temperature heat gun), cooled with a small sample flush (if necessary) and the microbiological samples are collected. Chemical samples, to be split for shipment to more than one laboratory, should be collected as a single sample, thoroughly mixed, and then split into the appropriate containers. Samples will be preserved as they are collected as recommended in **Section 3.1.4**. Sample collection labels should be affixed to each sample container and minimally contain the following information:

- NASA Sample collection number
- Time collected
- Initials of personnel collecting the sample
- How preserved
- Any anomalies encountered during sampling
- Laboratory to which the sample is to be sent
- Field tracking number
- Date collected
- Collection location/description
- Parameters for analysis

After collection, samples will be delivered to the Data Custodian for weighing **(see Appendix 4)** and recording into the Data Log (which resides on the FEDS database). Samples should be handled as little as possible after collection.

3.1.3 Sampling Tracking Procedures

Sample tracking procedures will be maintained for the sample life cycle. The sample life cycle will begin when the sample is collected and continues until final sample disposal. In addition, all records of sample tracking will be maintained for a minimum of seven (7) years following the end of the life cycle. Initial sample tracking is accomplished using sample labels and chain of custody forms generated by the FEDS. When it is not practical or possible to generate forms and labels by computer, these items will be filled out by hand just prior to sample collection by either the ACC, Data Custodian, or trained sampling personnel. Samples will be assigned a Sample Number and logged into the Data Log by the Data Custodian. Subsequent tracking of the samples will be accomplished as described in **Section 6.0.**

Each participating laboratory will assign a sample custodian and designate a sample storage area. Incoming samples will be received by the sample custodian and the appropriate chain-of-custody record signed.

Custody of samples within the laboratory is defined as:

- o In actual physical possession of authorized laboratory personnel
- o In view, after being in physical possession of authorized personnel
- o In secured storage to prevent tampering
- o In a secured area, restricted to authorized personnel

If a sample does not meet one of the above categories then it is not in custody[5]. If a sample must leave the primary laboratory, for any reason, it must be accompanied by a corresponding chain of custody **(see Appendix 4)**.

3.1.4 Sample Preservation and Storage

Sample degradation can begin immediately following collection. Preservation is necessary to retard the degradation of chemicals and/or the alteration of microbial populations in samples prior to analysis. Samples will be processed and relinquished by the Data Custodian within a maximum of six hours after collection. Samples should be analyzed in a timely manner as received by the laboratory. Sample aliquots which have holding times greater than 24 hours and which cannot be analyzed on the day of collection will be preserved and/or stored at 4° C. **Table 3-1** illustrates approved preservation, collection and storage containers, as well as acceptable holding times. NASA/MSFC requires that all sample analyses be completed within 28 days of laboratory receipt. Samples not analyzed within the required holding times, samples which have been inadequately preserved, or samples otherwise subjected to questionable conditions will be appropriately labeled and this information reported to the ACC. In addition, similar information should be contained in the FEDS, and easily accessable to all FEDS users. The ACC will flag these results as suspect. The ratings will be reported to the Principal Investigator through the PI (Principal Investigator) report generated by the FEDS.

All sample preservation will be accomplished at the time of collection unless otherwise noted in **Table 3-1**. Sample containers will be prepared with the appropriate preservative, sterilized if required, and labeled prior to use.

3.1.5 Sample Transport

Samples requiring overnight transport should be shipped on "blue ice" by an overnight delivery service. "blue ice" is used so that leakage will not occur and result in courier rejection. In additon, when used properly, blue ice will ensure that samples will be maintained at or below 4°C. Samples shipped to local analytical facilities may be transported on "blue ice" or regular ice, by NASA approved transportation services **(see Appendix 4)**.

3.1.6 Sample Storage

Samples will be stored by each participating laboratory under proper conditions in a controlled access facility **(see Table 3-1)**. In addition, the laboratory may be requested to freeze and archive representative samples for later use. Requests for such action will come from NASA, ED62, and/or the ACC.

3.1.7 Sample Disposal

Following review and analysis of the sample results and corresponding control data, each participating laboratory is authorized to dispose of their samples. The Principal Investigator will contact participating laboratories by way of written notification if samples should be retained. When all of a sample is used for analyses, the empty sample bottle may be disposed of prior to the review and analyses of sample results. When such action takes place, a record should be made of the disposal to cover any requests for additional data.

Table 3-1 RECOMMENDED PRESERVATION AND HOLDING TIMES FOR WATER SAMPLES[a,d]

Measurement	Allowable Container[b]	Preservative[c],	Max. Holding Time[e]
Physical Properties			
Color	P,G	Cool, 4°C	48 Hrs.
Conductance	P,G	Cool, 4°C	28 Days
Dissolved Gas	G only	NA	NA
Hardness	P,G	HNO_3 to pH<2	28 Days
pH	P,G	None Req.	None[f]
Filterable Solids	P,G	Cool, 4°C	7 Days
Non-Filterable Solids	P,G	Cool, 4°C	7 Days
Total Solids	P,G	Cool, 4°C	7 Days
Volatile	P,G	Cool, 4°C	7 Days
Settleable Matter	P,G	Cool, 4°C	48 Hrs.
Temperature	P,G	None Req.	None[f]
Turbidity	P,G	Cool, 4°C	48 Hrs.
Metals			
Dissolved	P,G	Cool, 4°C HNO_3 to pH<2	6 Mo.
Suspended	P,G	Cool, 4°C	6Mo.[g]
Total	P,G	HNO_3 to pH<2	6 Mo.
Chromium+6	P,G	Cool, 4°C	24Hrs
Sodium	P,G	Cool, 4°C[k] H_2SO_4 to pH 2	6 Mo.

Table 3-1 (continued) RECOMMENDED PRESERVATION AND HOLDING TIMES FOR WATER SAMPLES[a,d]

Measurement	Allowable Container[b]	Preservative[c],	Max. Holding Time[e]
Potassium	P,G	Cool, 4°C [k] H_2SO_4 to pH 2	6 Mo.
Mercury Dissolved	P,G	Filter HNO_3 to pH<2	28Days
Total	P,G	HNO_3 to pH<2	48 Hrs.
Inorganics, Non-Metallics			
Acidity	P,G	Cool, 4°C	14 Days
Alkalinity	P,G	Cool, 4°C	14 Days
Bromide	P,G	None Req.	28 Days
Chloride	P,G	None Req.	28 Days
Chlorine	P,G	None Req.	None[f]
Cyanides	P,G	Cool, 4°C NaOH to pH>12 0.6g ascorbic acid[h]	14 Days
Fluoride	P,G	None Req.	28 Days
Iodide	P,G	Cool, 4°C	24 Hrs.
Iodine	G only	Cool, 4°C zero head space	None[f]
Nitrogen			
Ammonia	P,G	Cool, 4°C [k] zero headspace: (EPA 350.2 & 300.7) H_2SO_4 to pH 2: (EPA 350.2 only)	 48 Hrs. 28 days
Kjeldahl, Total	P,G	Cool, 4°C H_2SO_4 to pH<2	28 Days

Table 3-1 RECOMMENDED PRESERVATION AND HOLDING TIMES FOR WATER (continued) SAMPLES[a,d]

Measurement	**Allowable Container**[b]	**Preservative**[c],	**Max. Holding Time**[e]
Nitrate plus Nitrite	P,G	Cool, 4^oC H_2SO_4 to pH<2	28 Days
Nitrate	P,G	Cool, 4^oC [i]	24 Hrs.
Nitrite	P,G	Cool, 4^oC	48 Hrs.
Dissolved Oxygen			
Probe	G bottle and top	None Req.	None[f]
Winkler	G bottle and top	Fix on site and store in dark	8Hrs.
Phosphorus			
Orthophosphate,	P,G	Cool, 4^oC	14 Days
Dissolved		Cool, 4^oC	
Hydrolyzable	P,G	Cool, 4^oC H_2SO_4 to pH<2	28 Days
Total	P,G	Cool, 4^oC H_2SO_4 to pH<2	28 Days
Total, Dissolved	P,G	Cool, 4^oC H_2SO_4 to pH<2	24 Hrs.
Silica	P only	Cool, 4^oC	28Days
Sulfur			
Sulfate	P,G	Cool, 4^oC	28 Days
Sulfide	P,G	Cool, 4^oC 2ml zinc acetate soln. plus NaOH to pH>9: added at analyzing lab	7 Days

Table 3-1 RECOMMENDED PRESERVATION AND HOLDING TIMES FOR WATER (continued) SAMPLES[a,d]

Measurement	Allowable Container[b]	Preservative[c],	Max. Holding Time[e]
Sulfite	P,G	None Req.	None[f]
Total Carbon	P,G	Cool, 4°C H_2SO_4 or HCl to pH <2	28 Days
Total Inorganic Carbon	P,G	Cool, 4°C H_2SO_4 or HCl to pH <2	28 Days
Organics			
BOD	P,G	Cool, 4°C	48 Hrs.
COD	P,G	Cool, 4°C	28 Days
Halogenate Hydrocarbons	G only	Cool, 4°C	7 Days
Oil & Grease	G only	Cool, 4°C H_2SO_4 to pH<2	28 Days
Organic Acids (all varieties)	G only	Cool, 4°C	NA
Organic Alcohols	G only	Cool, 4°C	NA
Organic Carbon			
total	P,G	Cool, 4°C H_2SO_4 or HCl to pH<2	28 Days
purgeable	G only	Cool, 4°C zero head space	7 Days
Pesticides	G only	Cool, 4°C	7 Days
Phenolics	G only	Cool, 4°C H_2SO_4 to pH<2	28 Days

Table 3-1 RECOMMENDED PRESERVATION AND HOLDING TIMES FOR WATER (continued) SAMPLES[a,d]

Measurement	**Allowable Container[b]**	**Preservative[c],**	**Max. Holding Time[e]**
Surfactants			
MBAS	P,G	Cool, 4°C	28 Days
NTA	P,G	Cool, 4°C	24 Hrs.
SDS	P,G	Cool, 4°C	24 Hrs.
NOPE	P,G	Cool, 4°C	24 Hrs.
SCMT	P,G	Cool 4°C	24 Hrs.
Toxic Organics			
BNA	G only	Cool, 4°C	7 Days
VOA	G only	Cool, 4°C Zero head space	14 Days
NVO	G only	Cool, 4°C	NA
Urea	G only	Cool, 4°C	NA
Microbiology			
Heterotrophic Plate Count	P sterile	Cool, 4°C $Na_2S_2O_3$, EDTA	24 hrs [j]
Aero-tolerant Eutrophic Mesophiles	P sterile	Cool,4°C $Na_2S_2O_3$, EDTA	24 hrs [j]
Fecal Coliforms	P sterile	Cool, 4°C $Na_2S_2O_3$, EDTA	24 hrs [j]
Yeast and Molds	P sterile	Cool, 4°C $Na_2S_2O_3$, EDTA	24 hrs [j]

Table 3-1 RECOMMENDED PRESERVATION AND HOLDING TIMES FOR WATER
(continued) SAMPLES[a,d]

Measurement	Allowable Container[b]	Preservative[c],	Max. Holding Time[e]
Total Count	P sterile	Cool, 4°C $Na_2S_2O_3$, EDTA	24 hrs [j]
		0.5% w/v glutaraldehyde	21 days
Gram Positives	P sterile	Cool, 4°C $Na_2S_2O_3$, EDTA	24 hrs [j]
Gram Negatives	P sterile	Cool, 4°C $Na_2S_2O_3$, EDTA	24 hrs [j]
Anaerobes (enumeration)	P sterile	Cool, 25°C $Na_2S_2O_3$, EDTA Cysteine	2 hrs
Anaerobes (qualitative)	P sterile	Cool, 25°C Thioglycollate	24 hrs [i]
Enterics	P sterile	Cool, 4°C $Na_2S_2O_3$, EDTA	24 hrs [j]
Legionellae	P sterile	Cool, 4°C $Na_2S_2O_3$	24 hrs [j]

a More specific instructions for preservation and sampling are found with each procedure as detailed in the EPA manual of Methods for Chemical Analysis of Water and Wastes. A general discussion on sampling water and industrial wastewater may be found in ASTM, Part 31, p. 72-82 (1976) Method D-3370.

b Plastic (P) or Glass (G). For metals, polyethylene with a polypropylene cap (no liner) is preferred.

c Sample preservation should be performed immediately upon sample collection. For composite samples each aliquot should be preserved at the time of collection. When use of an automated sampler makes it impossible to preserve each aliquot, then samples may be preserved by maintaining at 4°C until compositing and sample splitting is completed.

d When any sample is to be shipped by common carrier or sent through the United States Mail, it must comply with the Department of Transportation Hazardous Materials Regulations (49CFR Part 172). The person offering such material for transportation (ACC, Data Custodian, or other WRT team member) is responsible for ensuring such compliance.

e Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still considered valid. Samples may be held for longer periods only if the monitoring laboratory has data on file to show that the specific types of sample under study are stable for the longer time, and has received a variance from the Regional Administrator and NASA/MSFC, ED62. Some samples may not be stable for the maximum time period given in the table. A permittee, or monitoring laboratory, is obligated to hold the sample for a shorter time, if knowledge exists to show this is necessary, to maintain sample stability.

f There is no acceptable holding time established. Samples should be analyzed immediately upon collection.

g Samples should be filtered immediately on-site before the addition of preservative for dissolved metals.

h Should only be used in the presence of residual chlorine.

i For samples from non-chlorinated drinking water supplies conc. H_2SO_4 should be added to lower sample pH to less than 2. The sample should be analyzed before 14 days.

j Although eight (8) hours is given as a recommended holding time all samples should be processed as soon as practical. Microbiological populations and recovery are altered by increased storage times. Samples should not be analyzed if more than 24 hours elapse between collection and analysis.

k These samples may be preserved at the laboratory, but must be preserved within 48 hours of the collection time.

Source of Table: From EPA manual of Methods for Chemical Analysis of Water and Waste (Modified for use on the WRT).

3.2 Analytical Methods

3.2.1 Procedures

All methods used by the participating laboratories must be approved by the ACC, the Principal Investigator, MSFC Materials and Processing Laboratory, and the NASA/MSFC WRT Medical Monitor prior to use. The primary methods used are those outlined and approved by the Environmental Protection Agency (EPA) and the American Public Health Association (APHA). Secondary methods, when required, will be from recognized sources such as the Association of Official Analytical Chemists (AOAC) and the American Society for Testing Materials (ASTM). In some cases, recommended procedures or instrumental parameters for certain analyses are contained within a manufacturer's operating manual. Requests to utilize these methods on the WRT should be submitted to the ACC prior to use. **All modifications to any method as specified above must have prior written approval by the ACC, Principal Investigator, MSFC Materials and Processing Laboratory, and the Medical Monitor before use by a participating laboratory (also see Section 3.2.4).**

Efforts will be made to coordinate and standardize methodology between cooperating laboratories in order to eliminate methodology as a variable when comparing results and assessing validity and accuracy. **Table 3-2** lists currently approved methods for analysis. Any methodology must be thoroughly documented including precision and accuracy parameters for each analysis procedure within each participating laboratory.

3.2.2 Analyst Proficiency

Analysts who perform a particular method must demonstrate proficiency in and use of any instrumentation required. Refer to **Section 3.4.2.2**, Instrument Qualification and Training.

3.2.3 Detection Limits

Each laboratory must demonstrate the capability of meeting the required detection limits of each method as specified in **Table 3-2** unless such limits are waived (in writing) by the NASA/WRT PI.

3.2.4 Methods Development

It may be necessary to develop new methods for analyzing certain parameters. In addition, modification of existing methods may be necessary to improve recovery, account for matrix effects, or availability of instrumentation within a laboratory. For any new method or modification of an existing method, certain information must be obtained. First, side by side comparison with the existing method (if applicable) will be conducted for a minimum of seven individual samples. Second, validity, accuracy, and precision data must accompany the comparative results and will include, as a minimum, seven individual measurements **(Section 3.4.3.2)**. Third, complete written documentation of the procedure including steps outlining each aspect of the procedure as well as instrument settings and calibration data will be included. The complete documentation package is then submitted to the ACC. The ACC will verify the contents and forward copies of the data package to the Materials and Processing Laboratory (NASA/MSFC) and Biomedical Laboratories Branch (NASA/JSC). In addition, a copy of the method may be sent to an outside laboratory for verification. Written recommendations concerning the applicability of the method will be made to the Principal Investigator. A final determination will be made by the Principal Investigator, MSFC Materials and Processing Laboratory, and the Medical Monitor. If approved, the method will be added to the approved list. Copies of the approved method will then be sent to the participating laboratories for incorporation into their analytical program as applicable.

The following criteria[1,6] will be used to determine specific methods selected:

(1) The method will measure the desired constituents in the presence of normal interferences with required precision and accuracy.
(2) Each selected procedure will utilize available equipment and skills typically found in the laboratory.
(3) Each selected method will have been tested for established validity.
(4) The methods selected will be sufficiently rapid to allow for routine use for a large number of sample analyses.
(5) The methods selected will be capable of achieving the required detection limits.

Table 3-2 APPROVED PHYSICAL AND CHEMICAL ANALYTICAL METHODS

PARAMETER	METHOD	TECHNIQUE	UNITS	DET'N LIMIT[a]
PHYSICAL				
conductivity	EPA 120.1	specific conductance	umho/cm	1.0
color	SM 204 A SM 2120 B	spectrophotometric	units	1.0
dissolved gas	JSC Method	Syringe	NA	NA
odor	EPA 140.1	Threshold odor	TON	15
particulates		Climet CI-1000		
pH	EPA 150.1	Electrometric	pH units	0-14
total solids	EPA 160.3 SM 2540 B	Gravimetric	mg/l	10
total dissolved solids	EPA 160.2 SM 2540 C	Gravimetric	mg/l	10
total suspended solids	EPA 160.1 SM 2540 D	Gravimetric	mg/l	10
turbidity	EPA 180.1 SM 2130 B	Nephelometric	NTU	0
SG	SM 2710 F	Gravimetric	g/ml	NA
INORGANIC NONMETALS				
alkalinity	EPA 310.1 SM 2320 B	Titrimetric	mg/l as CaCO3	1.0
ammonia	EPA 300.7 Boeing In House	Ion chromatography	mg/l	0.05
bromide	SM 429 SM 4110 EPA 300.0 Boeing In House	Ion chromatography	mg/l	0.05
chloride	SM 429 SM 4110 EPA 300.0 Boeing In House	Ion chromatography	mg/l	0.04
chlorine, total residual	EPA 330.5 SM 4500 C1	Colorimetric	mg/l	0.1
fluoride	SM 429 SM 4110 EPA 300.0 EPA 340.2 Boeing In House	Ion chromatography	mg/l	0.02
iodide	SM 414 A SM 4500 IB	Leuco crystal violet	mg/l	0.1
iodine, total	SM 414 A SM 4500 IB	Leuco crystal violet	mg/l	0.1
iodine, free	EPA 300.0 SM 4500 IB	Leuco crystal violet	mg/l	0.1
nitrate	SM 429 SM 4110 EPA 300.0 Boeing In House	Ion chromatography	mg/l	0.1
nitrogen, total	SM 420 EPA 351.1	Colorimetric	mg/l	1.0

Table 3-2 APPROVED PHYSICAL AND CHEMICAL ANALYTICAL METHODS (continued)

PARAMETER	METHOD	TECHNIQUE	UNITS	DET'N LIMIT[a]
phosphate	SM 429 SM 4110 EPA 300.0 Boeing In House	Ion chromatography	mg/l	0.3
sulfate	SM 429 SM 4110 EPA 300.0 Boeing In House	Ion chromatography	mg/l	0.15
sulfide	AU 107	Ion chromatography	mg/l	0.05
total carbon	SM 505 B SM 5310 B	UV persulfate/NDIR	mg/l	0.2
total inorganic carbon	SM 505 B SM 5310 B	UV persulfate/NDIR	mg/l	0.2
INORGANIC METALS				
arsenic	EPA 206.2 EPA 200.7	AAG	mg/l	0.002
barium	EPA 200.7	ICP	mg/l	0.002
	EPA 208.2b	AAG	mg/l	0.002
cadmium	EPA 200.7	ICP	mg/l	0.01
calcium	EPA 200.7	ICP	mg/l	0.01
chromium	EPA 200.7	ICP	mg/l	0.01
	EPA 218.2b	AAG	mg/l	0.01
copper	EPA 200.7	ICP	mg/l	0.01
	EPA 220.2b	AAG	mg/l	0.01
iron	EPA 200.7	ICP	mg/l	0.02
	EPA 236.2b	AAG	mg/l	0.02
lead	EPA 239.2 EPA 200.7	AAG	mg/l	0.001
magnesium	EPA 200.7	ICP	mg/l	0.03
	EPA 242.1b	AAF	mg/l	0.03
manganese	EPA 200.7	ICP	mg/l	0.005
	EPA 243.2b	AAG	mg/l	0.005
mercury	EPA 245.1	Hg analyzer	mg/l	0.0002
nickel	EPA 200.7	ICP	mg/l	0.03
potassium	EPA 300.0	Ion chromatography	mg/l	0.05
selenium	EPA 270.2	AAG	mg/l	0.002
silver	EPA 200.7	ICP	mg/l	0.03
sodium	EPA 300.0	Ion chromatography	mg/l	0.02
zinc	EPA 200.7	ICP	mg/l	0.01
	EPA 289.2b	AAG	mg/l	0.01

Table 3-2 APPROVED PHYSICAL AND CHEMICAL ANALYTICAL METHODS (continued)

PARAMETER	METHOD	TECHNIQUE	UNITS	DET'N LIMIT[a]
ORGANICS				
total organic carbon	SM 505 B	UV persulfate/NDIR	ug/l	200
low level total organic carbon	EPA 415.2 L	UV persulfate/NDIR	ug/l	
specific organics:				
acid extractables	EPA 625/525c series	GC/MS	ug/l	note d
base/neutral ext.	EPA 625/525c series	GC/MS	ug/l	note d
volatiles	EPA 624/524c series	GC/MS	ug/l	note d
nonvolatiles	Boeing In House	GC/MS	ug/l	note d
phenols	EPA 625/525c series SM 5530	GC/MS	ug/l	note d
cyanide, free	AU 107	Ion chromatography	ug/l	20
cyanide, total	EPA 335.2	Spectrophotometric	ug/l	20
halogenated hydro-carbons	EPA 625/525c	GC/MS	ug/l	note d
organic acids	TBD	GC	ug/l	note d
organic alcohols	Boeing In House (tentative)	GC/FID Head space	ug/l	0.2
pesticides	EPA 608/508c	GC/ECD	ug/l	note d
volatile fatty acids	Boeing In House (tentative)	GC	ug/l	note d
nonvol. fatty acids	Boeing In House (tentative)	GC	ug/l	note d
semivol. fatty acids	Boeing In House	GC	ug/l	note d
herbicides	SM 6640 B	GC/MS	ug/l	note d
aldehydes	Boeing In House (tentative)	GC	ug/l	note d
MISCELLANEOUS				
urea	Boeing In House	HPLC	ug/l	100
methylene blue active substances (MBAS)	EPA 425.1 SM 5540 C	Colorimetric	ug/l	100
SCMT	Boeing In House (tentative)	NA	NA	NA
nonyl (9-10)	Boeing In House (tentative)	NA	NA	NA
Na dodecyl benz. sulf.	Boeing In House (tentative)	NA	NA	NA

[a]The detection limits listed represent either the limit or the applicable range stated in the respective method. For those methods for which detection limits or applicable ranges are not defined, the limits listed in this table reflect the minimum detection limit anticipated to be required to meet phase III objectives.

[b]Secondary method

[c]For future tests, series 500 methods for drinking water may be required.

[d]Species-specific

3.3 Interlaboratory Control

The interlaboratory control program is a systematic testing program in which uniform samples are analyzed by all participating laboratories to assess the continuing capability, relative performance, and improvement in identified weak areas of each lab. The purposes of interlaboratory testing are as follows:

- o To provide a measure of the precision and accuracy of analytical methods run routinely by participating laboratories
- o To estimate the accuracy and precision of results between laboratories
- o To identify weak methodology
- o To identify inadequate equipment and instrumentation
- o To detect training needs
- o To upgrade the overall quality of the laboratory performance

3.3.1 Analytical Control Samples

Analytical control samples will consist of initial qualification samples, and periodic verification, blind duplicate, and split set samples. All analytical control samples, except for the blind duplicates, will be purchased by the ACC and certified by the vendor for authenticity. Efforts will be made to mimic individual matrices and analysis parameters where applicable.

3.3.2 Initial Qualification

Initially each participating laboratory will be sent a series of three (3) qualification check samples. Each participating laboratory will be identified by a laboratory code so that performance results may remain anonymous to other participants. Qualification samples will be certified, and will include a wide range of analytes so that performance can be determined for individual classes of compounds. Tentative parameters and ranges of analyte concentrations are presented in **Table 3-3**. Results of the completed analyses will be reported directly to the ACC within two weeks of receipt. Performance results of each laboratory will in turn be reported to the Principal Investigator and the MSFC Materials and Processing Laboratory representative within the following two weeks. The ACC will report the results of the qualification samples directly to the individual laboratories immediately following the review. If an area is identified as weak for a participating laboratory, a plan to correct these deficiencies must be submitted to the ACC prior to continued participation by that laboratory. Subsequent results provided in an identified deficient area will be labeled as "suspect" until the deficiencies are corrected. Improvement will be monitored through the qualification program. If an area deficiency is not corrected by the third qualification sample, the laboratory will be restricted to only those analyses for which it demonstrated proficiency or excluded from further participation entirely. **The decision to restrict or eliminate a laboratory from participation will be made by the Principal Investigator, MSFC Materials and Processing Laboratory, and/or the NASA/MSFC WRT Medical Monitor.**

Table 3-3 TYPICAL PARAMETERS AND CONCENTRATIONS FOR QUALIFICATION, VERIFICATION, AND REFERENCE SAMPLES

PARAMETER	CONCENTRATION RANGE
Physical	
conductivity	500-2900 umho/cm
pH	5-10 pH units
Total solids	500-2000 mg/l
total dissolved solids	500-2000 mg/l
total suspended solids	10-120 mg/l
turbidity	0.3-10 NTU
Inorganic Nonmetals	
alkalinity	100-300 mg/l as CaCO3
ammonia	1-20 mg/l
chloride	50-400 mg/l
chlorine, total residual	0.5-3 mg/l
fluoride	1-20 mg/l
nitrate	1-20 mg/l
nitrogen, total	1-20 mg/l
phosphate	1-10 mg/l
sulfate	50-400 mg/l
Inorganic Metals	
arsenic	10-250 ug/l
barium	20-2000 ug/l
cadmium	5-500 ug/l
calcium	10-200 mg/l
chromium	20-2000 ug/l
copper	20-2000 ug/l
iron	20-2000 ug/l
lead	20-2000 ug/l
magnesium	5-200 mg/l
manganese	20-2000 ug/l
mercury	1-20 ug/l
nickel	20-2000 ug/l
potassium	10-300 mg/l
selenium	5-500 ug/l
silver	5-500 ug/l
sodium	10-300 mg/l
zinc	50-1000 ug/l

Table 3-3 TYPICAL PARAMETERS AND CONCENTRATIONS FOR
(continued) QUALIFICATION, VERIFICATION, AND REFERENCE SAMPLES

PARAMETER	CONCENTRATION RANGE
Organics	
total organic carbon	10-100 mg/l
specific organics:	
acid extractables	10-250 ug/l
base/neutral ext.	10-250 ug/l
volatiles	1-200 ug/l
phenols	25-500 ug/l
cyanide	25-500 ug/l
halogenated hydrocarbons	0.01-10 ug/l
halomethanes	1-100 ug/l
pesticides	0.01-10 ug/l

3.3.3 Verification Samples

Verification samples will be provided to the participating laboratories on a periodic basis. The purpose of the verification samples will be to monitor the continual performance of the participating laboratories and provide a mechanism for monitoring improvement of any identified deficient area(s) within a participating laboratory. The verification samples will be treated as a supplement to the qualification samples. The samples will consist of certified, purchased samples containing a wide variety of analytes as previously identified **(Table 3-3)**.

3.3.4 Blind Samples (Control Samples)

Blind samples are those samples which are prepared by someone other than the analyst or laboratory performing the work. With the exception of the duplicate samples, all blind samples submitted to the participating laboratories will be certified for authenticity by the vendor, and may have cross verification by other independent laboratories. The blind samples will be used as a independent check for accuracy and precision. Blind samples will be submitted to the participating laboratory, during testing, as part of the overall sampling regime.

3.3.4.1 Duplicate Samples

At random time intervals, blind duplicate samples will be collected or prepared and submitted to each laboratory. Blind duplicate samples may be either process samples collected and submitted as two separate samples within a single batch or certified (verified) reference samples which are split and submitted as two separate samples within a given day. These samples will be used by the ACC to monitor precision of the overall laboratory procedure. Blind duplicate samples may be submitted as additions to the test sample sets **(Section 3.3.4.2)** or with reference sample sets **(Section 3.3.4.3)**.

3.3.4.2 Split Samples

At random time intervals, samples collected will be split and submitted to two or more participating laboratories. These samples will be used to determine the precision between the individual laboratories for specific method parameters. To ensure compatibility of laboratories within this program, all participating laboratories are instructed to use only those methods approved in **Table 3-2** when performing such analyses, and all WRT analyses.

3.3.4.3 Reference Samples

At random time intervals, blind reference samples will be submitted to the individual participating laboratories. Reference samples may be submitted in duplicate or singly. These samples will be used by the ACC as a check of precision and/or accuracy for individual parameters. The parameters and concentration ranges of the blind reference samples will be as previously identified **(Table 3-3)**.

3.3.5 Reference Laboratory

Reference laboratories may be identified based on experience, recognition, and demonstrated performance in specific areas. Samples chosen at random as well as any samples labeled as "key samples" (by the PI or Medical Monitor) will be sent to reference laboratories for verification. "Key samples" are those samples identified by either the PI or Medical Monitor to be of primary importance to the outcome of the test, or test subject safety.

3.3.6 Laboratory Audits

Both formal and informal laboratory audits will be conducted during the course of the WRT. Informal audits may consist of personal or telephone interviews with individual laboratory personnel or the Directors/Managers of the participating laboratories. Informal audits need not be announced and will generally be used to discuss specific problems, individual results submitted, or methods used by the laboratory. The informal audits will be conducted by the ACC **(see Appendix 5)**.

Formal audits will consist of both off-site and on-site inspection activities. Formal audits will be announced and coordinated with the Director/Manager of the participating laboratory. Results and suggestions resulting from all formal audits will be provided to the individual Laboratory Director/Manager in writing. Formal audits will be conducted by two or more individuals representing the ACC, the PI, Medical Monitor and the MSFC Materials and Processing Laboratory.

3.3.6.1 Off-Site Inspections

Off-site inspections will consist of review of laboratory documents, raw data, quality assurance data, protocols, sampling plans, and any other material deemed pertinent by either the PI, Medical Monitor, MSFC Materials and Processing Laboratory or the ACC. The review may take place with or without laboratory personnel involvement. The site inspections will be conducted as deemed appropriate by the ACC.

3.3.6.2 On-Site Inspections

On-site inspections will be conducted periodically by an audit team. Notification will be given to the Laboratory Director/Manager the week before the inspection. On-site inspections will be conducted at random or if a significant problem is suspected within the laboratory. Generally, routine audits will be conducted for each participating laboratory initially and then twice per year (either formal or informal as deemed appropriate by the ACC). An on-site inspection will be conducted and will last no more than two working days and will be structured such that it will have minimal effect on the normal laboratory operation. During the inspection, the audit team will interview laboratory personnel, management personnel, and the Laboratory Control Coordinator. They will also inspect record-keeping procedures, sample handling and control procedures, analyses procedures, laboratory safety, instrumentation, staff qualifications and experience, workload, and understanding of analysis procedures.

Upon conclusion, an oral debriefing will be made to the Laboratory Management outlining the findings. A final written report will be prepared outlining problems noted and subsequent recommendations.

3.3.7 Laboratory Certification

With the exception of the participating clinical laboratories, laboratory certification or accreditation is not a requirement for participation at this time. However, each participating laboratory is strongly encouraged to pursue Safe Drinking Water Act (SWDA) certification independently. Certification or accreditation programs help to improve and maintain the validity, accuracy, and precision of test data and promote the acceptance of test data by the users. In addition, test data produced by certified or accredited laboratories are more readily accepted by other laboratories without further testing. **It should be noted that laboratory certification for participation in recipient mode testing or analysis of samples relating to donor safeguards may be a requirement.**

3.4 Intralaboratory Control

Intralaboratory control describes those procedures used within a given laboratory to produce and maintain quality results. An adequate intralaboratory control program provides a continuing measurement of performance of individual analysts as well as instrumentation.

3.4.1 Laboratory Documentation

All operations, procedures, methodologies, and reporting pertinent to the laboratory should be thoroughly documented for both management and laboratory personnel.

3.4.1.1 Standard Operating Procedures Document

Each participating laboratory will have a written Standard Operating Procedures (SOP) document. As a minimum the laboratory SOP document should address the following:

- o Personnel
- o Organization and Management
- o Special Situations and Emergencies (who to contact)
- o Facilities and Services
- o Instrumentation
- o General Procedures
- o Analytical Methods
- o Log of personnel who have read the document

3.4.1.2 Analytical Control Document

Each participating laboratory will have a written Analytical Control Document. As a minimum the AC document should address the following:

- o Sample Collection and Handling
- o Sample Tracking
- o Quality Control Procedures
- o Quality Assurance Procedures
- o Data Handling and Reporting
- o Log of personnel who have read this document

3.4.2 Personnel

3.4.2.1 Skill Level

The skill level of personnel and understanding of relevant procedures and instrumentation are essential to providing quality results in an analytical laboratory. Lead chemists should possess an advanced degree and/or have written approval for the position from NASA, MSFC. Staff chemists should possess a degree in chemistry. Ideally, the program of study should be accredited by the American Chemical Society (ACS). Laboratory technicians in the chemistry laboratory should **minimally** possess a high school diploma and have received thirty days on the job training which should include specific instruction on the procedure or instrumentation he/she is expected to operate (BS in Chemistry is preferred). Minimal guidelines have been established in order to relate necessary skill level requirements to the complexity of the analytical and instrumental procedures involved [1]. These guidelines are presented in **Table 3-4**. All laboratory personnel should be supervised by an experienced professional scientist.

Table 3-4 SKILL RATING FOR STANDARD ANALYTICAL OPERATIONS[1]

MEASUREMENT	SKILL REQUIRED
Simple Instrumentation	
pH, Conductivity, Turbidity, Color	1
Dissolved Oxygen	1,2
Simple Volumetric	
Alkalinity, Acidity, Chloride, Hardness	1
Simple Gravimetric	
Solids	1,2
Simple Colorimetric	
Nitrate, Nitrite, Sulfate, Silica	2
Complex Gravimetric or Colorimetric	
BOD, COD, TKN, Ammonia, Oil and Grease, Fluoride, Cyanide	2,3
Special Instrumentation or Procedure	
TOC	2,3
Inorganic non-metals (Ion Chromatography)	2,3
Metals (Atomic Absorption)	2,3
Metals (Inductively Coupled Plasma)	2,3
Organics (Gas Chromatography)	3,4
Organics (Liquid Chromatography)	3,4
Methods Development	3,4

1 semi-skilled

2 experienced aide or a professional with modest training and experience

3 professional with good background and experience in analytical techniques

4 professional with specialization, requires interpretation of results

3.4.2.2 Instrument Qualification and Training

It is generally recognized that for experienced, higher grade personnel, formal training in special fields, techniques, and instrumentation is a mandatory requirement. Similar formal training for lower grade personnel is not readily available. For these individuals an in-house training program should be established to improve analytical capabilities, conceptual understanding, instrumental procedures, and quality performance. This can be accomplished through an established program of working with more experienced analysts, cross training, laboratory seminars, and exposure to pertinent literature.

Notification of any changes to the laboratory section leaders within a participating laboratory must be made directly to the ACC. The ACC will then notify the PI, MSFC Materials and Processing Laboratory's representative and the NASA/MSFC WRT Medical Monitor of these changes.

3.4.2.3 Laboratory Control Coordinator

Each participating laboratory is expected to designate a single person separate from the analysis personnel whose sole responsibility is surveillance and monitoring of in-house analytical control activities. This person will have direct contact with the ACC, and will be the contact point, at the participating laboratory, for all ACC activity.

3.4.3 Analytical Controls

The terms Quality Assurance (QA) and Quality Control (QC) refer to the whole spectrum of laboratory practices designed to monitor and assure accuracy, precision, and validity of the results measured. In analytical laboratories which are not involved in industrial processes, the following definitions are generally accepted. QC refers to special procedures for demonstrating the validity of results. The primary objective of QC is to provide a system of activities to assure the quality of the analyses. QA refers to all elements of proper technique which minimize errors. Therefore, the goal of QA is to provide a system of activities to assure that the QC system is performing properly by meeting the program requirements for reliability.

The laboratory QA/QC program will encompass and support all laboratory operations which include sample collection (if performed by the laboratory), sample tracking, analytical methods, instrumentation, policies, and documentation/reporting activities. It is essential that standard written practices be established within the laboratory to promote efficient and effective operation and to assure that QA/QC program procedures are incorporated into the operational structure. Analytical control must begin with sample collection and must not end until the resultant data have been reported. Conscientious use and understanding of analytical control measures among field, analytical, and management personnel is imperative. Because of the importance of laboratory analyses in determining practical courses of action that may be followed, quality assurance programs, to insure the reliability of the water and wastewater data, are essential.

The QA/QC program should facilitate the following specific objectives:

(1) Define the responsibilities of laboratory personnel associated with a given project.

(2) Associate reliability estimates for the level of quality associated with any analytical method, system, or process.

(3) Assist in the early recognition of deficiencies or problems which might affect data quality.

(4) Enable the Laboratory Control Coordinator to take corrective action as required to insure the validity of laboratory data.

(5) Enhance the utility of all data by requiring adequate documentation to support anticipated decision-making.

(6) Provide an organizational plan which may be used to implement quality control in laboratory operations.

3.4.3.1 Intralaboratory Control Samples

With each batch of analyses the following samples will be included as a minimum:

Prior To Analysis Of Unknowns

1	water, reagent or solvent blank, as applicable
1	preservative blank, if applicable
1	method blank
1	reference sample
3-5	standards Interspersed With Unknown Samples
1	spiked sample for every 20 unknowns or per analysis batch
1	set of duplicate samples for every 20 unknowns or per analysis batch
1	standard for every 20 unknowns

Following Analyses of Unknowns

1	reference sample

Each of these samples are described in the following sections.

3.4.3.2 Definitions

The following definitions have been suggested by the EPA in order to standardize their use[13].

3.4.3.2.1 Internal Standard

A pure analyte added to a solution in known amount and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.4.3.2.2 Surrogate Analyte

A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.4.3.2.3 Laboratory Duplicates (LD1 and LD2)

Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 provide a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4.3.2.4 Field Duplicates (FD1 and FD2)

Two separate samples collected at the same time, placed under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.4.3.2.5 Laboratory Reagent Blank (LRB)

An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.4.3.2.6 Field Reagent Blank (FRB)

Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.4.3.2.7 Laboratory Performance Check Solution (LPC)

A solution of one or more compounds used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.4.3.2.8 Laboratory Fortified Blank (LFB)

An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.4.3.2.9 Laboratory Fortified Sample Matrix (LFM)

An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.4.3.2.10 Stock Standard Solution

A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.4.3.2.11 Primary Dilution Standard Solution

A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.4.3.2.12 Calibration Standard (CAL)

A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.4.3.2.13 Quality Control Sample (QCS)

A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

3.4.3.3 Control Charts

Quality control charts are developed to evaluate validity, accuracy and precision. It is inherent that variations exist in every method. Where variations do not seem to exist, either the device used for measuring the process may not be sensitive enough, or the analyst may not be performing a procedure properly.

The emphasis for conducting accuracy and precision checks and maintaining quality control charts is to ensure and document the continuing validity of laboratory data. Therefore, it is imperative that analytical control measures be applied daily to provide constant quality control monitoring.

Valid accuracy and precision data must be developed for each analyst using a particular method and specific instrumentation. Poor accuracy data may result from analytical errors such as inaccurate dilution techniques, incorrect volume/weight measurements, or improper equipment calibration. Low precision is likely to result from low instrument sensitivity or other factors beyond the analyst's control. Therefore, a situation may exist where accuracy is high, but precision is low, or vice versa precision may be high and accuracy low. From the time a sample is logged into the laboratory until the data is reported, the analyst has the responsibility to perform all work in a skillful manner to provide high precision and accuracy within the scope of his/her control for all sample handling and analysis procedures.

3.4.3.3.1 Validity

To ensure that valid data is continually produced, systematic daily checks must show that the test results are reproducible, and that the methodology is actually measuring the quantity of the specified constituent in each sample. Validity checks are performed by analysis of a known reference sample for each parameter immediately preceding the analysis of unknown samples. Validity checks cannot be performed for methods for which no standard solution can be obtained. These methods include certain physical characteristics and microbiological procedures.

The accepted concentration value and upper and lower control limits are plotted over time. Acceptable control limits are defined as the accepted concentration value plus and minus three standard deviations, where fifty percent or greater of the determinations fall within one standard deviation.

A reference sample will be analyzed for each parameter prior to analysis of unknown samples and concentration values plotted on the control chart. The laboratory name, analysis number, parameter, method, sample concentration range, analyst's name, and date will be identified on the control chart. Control charts will be reviewed and maintained by the Laboratory Control Coordinator.

If analysis of reference samples reveals an out-of-control situation, i.e. a value falls outside the control limits, the problem will be identified and resolved prior to analysis of unknown samples.

3.4.3.3.2 Accuracy

Accuracy refers to the agreement between the amount of a constituent measured by a particular method and the actual amount of the constituent present in the sample. There are two categories of samples employed for evaluating accuracy. The first of these entails the use of reference samples previously described in **Section 3.4.3.3.1**. The second category incorporates the use of spiked samples in which a known quantity of a reference standard containing a particular constituent is added to a test sample.

Accuracy cannot be determined for certain methods where reference standards are not available, and therefore cannot be added in known quantities to test samples for percent recovery determinations.

Accuracy control charts are established to evaluate the data obtained from reference and spiked samples. For both the reference samples and the spiked samples, the percentage recovery is calculated and plotted.

A linear relationship exists between the percentage recovery and the known concentrations of standards and spikes[7]. The data from reference samples and spiked samples should be collected and percent recovery calculations made for a minimum of twenty analyses in order to establish control chart parameters. The control chart is not valid if less than fifty percent of the initializing data fall within the accepted value plus and minus one standard deviation. In addition, none of the initializing data may fall outside the upper and lower control limits[1]. Accuracy control charts will be identified by the laboratory name, parameter, method, sample concentration range, analyst, and date. Control charts will be reviewed and maintained by the Laboratory Control Coordinator.

3.4.3.3.3 Precision

Precision refers to the reproducibility of a method when it is repeated in a homogenous sample, regardless of whether or not the observed concentrations are representative of the true concentrations. Since precision is dependent upon concentration, control charts will be developed or applied within limited concentration ranges.

Duplicate samples are prepared and analyzed to evaluate precision, excluding results below the detection limit, and including analyses of the same reference sample performed on the same day. Precision can be monitored for individual analysts or between analysts. Precision can be measured for all methods. The control chart is not valid if less than fifty percent of the initializing data fall within the accepted value plus or minus one standard deviation. In addition, none of the initializing data may fall outside the upper and lower control limits of three standard deviations[1].

Precision control charts will be identified by the laboratory name, parameter, method, concentration range, analyst, and date. Control charts will be reviewed and maintained by the Laboratory Control Coordinator.

4.0 REQUIREMENTS FOR MICROBIOLOGICAL ANALYSIS OF WATER AND AIR

Microbiological parameters measure living organisms which continually change over time and in response to environmental conditions. Because absolute values do not exist for microbiological parameters, spiked samples cannot be prepared for routine evaluations of accuracy within the microbiology laboratory. Therefore, a Laboratory Control Program for microbiological analysis must address the control of laboratory operations, analytical procedures, and analyst precision. Subsequently, control procedures governing sample collection and handling, personnel, facilities, methodology, supplies, and equipment must be continuously monitored[1]. In addition, validity checks, positive and negative controls, sterility checks, replicate analysis, and the use of verification/confirmation procedures must be used to provide analytical controls.

4.1 General Operations

4.1.1 Laboratory Organization and Management

The microbiology laboratory's organization and management must be clearly defined. Requirements and responsibilities for each management and staff position will be documented. The laboratory will maintain a current SOP document specific to the microbiology laboratory describing approved procedures and techniques to be followed by all personnel.

As a minimum, the SOP document for the microbiology laboratory should address the following:

- o Facilities and Personnel
- o Sample Collection and Handling
- o Laboratory Equipment and Instrumentation
- o Laboratory Supplies
- o Media and Reagent Preparation
- o Reference Cultures
- o General Procedures
- o Analytical Methods
- o Analytical and Contamination Control Procedures
- o Log of personnel who have read the document

4.1.2 Laboratory Personnel

The microbiology laboratory should have similar personnel and professional levels as found in a college microbiology program. Lead microbiologists should possess an advanced degree and/or equivalent experience. Staff microbiologists should possess a degree from an accredited institution and have met certain course requirements[8]. In addition, staff microbiologists should have a minimum of one year bench experience in sanitary (water, milk or food) microbiology and received a minimum of two weeks supplemental training for each laboratory procedure he/she is expected to perform[5]. Ideally, staff microbiologists should be certified by the American Academy of Microbiology (AAM) or the American Society of Clinical Pathologists (ASCP). Laboratory technicians in the microbiology laboratory should minimally possess a high school diploma and have received 30 days on the job training including specific instruction in aseptic technique and safety considerations relating to biological hazards. Laboratory personnel should be supervised by an experienced professional scientist.

4.1.3 Laboratory Facilities

The laboratory facilities must be neat and organized into appropriate work stations. The space allotted for microbiological procedures must be adequate for the work load and number of employees assigned to this area. A minimum of six (6) linear feet of bench space for each analyst assigned to the laboratory is recommended[5].

4.1.4 Laboratory Cleanliness

High standards for cleanliness must be maintained in microbiological work areas. This is imperative not only for protection of the worker but also for protection of samples from outside contamination. A routine program should be implemented and monitored to minimize and identify potential contamination sources of both the laboratory and samples. This program should include scheduled cleaning and disinfection, monitoring UV lamp intensity, and monitoring water, surface, and air contamination within the laboratory.

4.1.5 Record-keeping

Written documentation of all laboratory practices and activities is imperative to adequate laboratory control. This is accomplished by maintaining activity records and log books documenting samples received by the laboratory, specific laboratory procedures and practices, verification of proper equipment operation, results of positive and negative controls, sterility checks, validity checks, and replicate analyses.

4.2 Instrument Use Procedures

Instruments and equipment used in the laboratory must be kept in good working order and operated properly. This section describes the controls for maintaining and verifying that all laboratory instrumentation and equipment is in good working order.

4.2.1 Preventative Maintenance

A regular program for scheduled maintenance of instruments and equipment will be maintained by the laboratory.

4.2.2 Validation of Proper Operation

All equipment must be validated, during use, that it is performing satisfactorily and within control limits set by the procedure being performed or the manufacturer's specification, as applicable.

4.2.3 Calibration

All instrumentation and equipment, in use within the laboratory, will be calibrated on a regular basis.

4.3 Special Equipment

The microbiology laboratory has specific and special equipment requirements. Procedures for the proper operation of this equipment should be included in the microbiology laboratory's SOP document. The minimum requirements for an adequately equipped microbiology laboratory are listed below. Due to the diverse nature of microbiological parameters which may be monitored by a given laboratory, no recommendation on the specific type and quantity of equipment is specified herein. Specific equipment requirements are identified in the referenced methodologies.

The minimum equipment requirements for a participating laboratory to conduct specific microbiological analyses include:

- autoclave
- pH meter with temperature probe
- vortex mixers
- gram staining equipment
- microbial identification equipment
- analytical balance
- hot plate/stirrer
- drying oven
- refrigerator
- freezer
- incubators
- anaerobic chambers
- water baths
- membrane filtration assembly
- UV sterilization unit
- vacuum pump w/water trap
- laminar flow hood
- class II biological safety cabinet
- water purification system
- microscopes

4.4 Methods and Procedures

4.4.1 Sample Collection and Handling (Refer to Section 3.1).

4.4.2 Preparation of media and reagents

4.4.2.1 Water

Water quality in the microbiology laboratory is of extreme importance. **Table 4-4** lists the NASA/MSFC WRT acceptable limits of water quality for microbiological analysis and the frequency of verification.

Table 4-1 WATER QUALITY REQUIREMENTS AND MONITORING FREQUENCY FOR MICROBIOLOGICAL ANALYSES[5]

ANALYSIS PARAMETERS	MONITORING FREQUENCY	LIMIT
Chemical Tests		
Conductivity	Continuously	<2 umhos/cm
pH	Each Use	5.5-7.5
TOC	Monthly	<1 mg/L
Heavy Metals		
(Cd, Cr, Cu, Ni, Pb & Zn)	Monthly	<0.5 mg/L
(total)	Monthly	<1 mg/L
Ammonia/Organic Nitrogen	Monthly	<0.1 mg/L
Residual Chlorine	Monthly	Not Detectable
Bacteriological Tests:		
Heterotrophic Plate	Counts Weekly	<1000 CFU/mL
Water Quality Tests	Annually	0.8-3.0 ratio
Use Test	Annually	Student's t < 2.78

4.4.2.2 Reagents

Only chemicals of ACS reagent grade or equivalent may be used. Impurities may provide growth promoting or growth inhibiting effects or interfere with the desired reaction. All chemicals should be labeled with date received and date opened. Opened chemicals should be stored in a cool dry place unless otherwise noted and should not be kept for more than one year. Reagents should be prepared using Class A (or calibrated) volumetric flasks and transferred to appropriate containers for storage. Prepared reagents will be labeled with name, concentration, date prepared, preparer's initials, and expiration date.

4.4.2.3 Culture Media

The isolation, recovery and maintenance of microorganisms depends upon the quality of ingredients and the proper preparation of the culture media. If available, commercially formulated media will be used. Unopened bottles of media will be stored in a cool dry place and may be kept for up to two years. Culture media should be dated when received and when opened for use. Opened bottles of media may be stored for up to one year. Culture media should be prepared as directed by the supplier. Media should be dissolved in deionized or distilled water **(see Section 4.4.2.1)** using heat and continuous stirring. Bring media to a gentle boil and remove from heat immediately to avoid scorching. If required, autoclave media for the minimum time specified. The pH of the media should be checked and adjusted for every batch. Media must be allowed to cool. Sterility checks and positive and negative control checks are made for each batch of medium. All information is recorded in the media preparation log book. Prepared media may be stored only for a finite time[5]. **Table 4-2** lists the recommended shelf life for prepared media. All prepared media will be stored in the dark at 4°C until use. Culture media must be allowed to equilibrate to room temperature before use to prevent temperature shock of the microorganisms. Under no circumstances may media be reautoclaved and used.

Table 4-2 RECOMMENDED SHELF LIFE FOR PREPARED MEDIA[5]

MEDIA TYPE	SHELF LIFE
Membrane filter (MF) broth in screw-cap flasks at 4 °C	96 hours
MF agar in plates with tight fitting covers at 4 °C	2 weeks
Agar or broth in loose-cap tubes at 4 °C	1 week
Agar or broth in tightly closed screw-cap tubes at 4 °C	3 months
Poured agar plates with loose fitting covers in sealed plastic bags at 4 °C	2 weeks

4.4.3 Glassware

Special precautions and procedures must be followed in the cleaning and preparation of glassware for use in microbiological determinations. All glassware should be examined and chipped or badly etched glassware should be discarded. Extra care must be taken to insure that growth promoting or inhibitory compounds are removed prior to use. Several methods are available and recommended for verification of glassware before use[5]. These include:

- o visual inspection of excessive water beading
- o acid or alkaline residues
- o residual detergent
- o growth promotion/inhibition

4.4.4 Plasticware

Plasticware, when used, should be presterilized and disposable.

4.4.5 Analytical Methods

The analytical methods for the analysis of microbiological parameters are presented in **Table 4-3.** Substitution or modification of any listed method must have the prior written approval by the ACC, PI, MSFC Materials and processing Laboratory, and the NASA/MSFC WRT Medical Monitor **(see Section 3.2.4).**

4.4.6 Specialized Procedures

Occasionally specialized procedures may be required and conducted by participating laboratories. These include microbiological identification, anaerobe isolation and enumeration, isolation and identification of pathogenic microorganisms, detection and enumeration of viruses, and the detection, isolation, and enumeration of biofouling organisms. For these cases, complete written documentation of the procedures to be followed is required **(Section 3.2.4)**. In addition, the skill level of personnel required for these tasks will be those outlined for Lead microbiologists **(Section 4.1.2)**.

4.5 Analytical Control Procedures (In House)

4.5.1 Positive and Negative Controls

Positive and negative control organisms (ATCC reference cultures) must be used in every phase of laboratory operation. This would include the preparation of culture media, determining physiological and biochemical characteristics, selective isolation and enumeration procedures, and identification of isolates. The proper use of reference cultures must be described and documented.

4.5.2 Replicate Analysis

For enumeration of microorganisms from unknown samples, all sample aliquots and dilutions will be analyzed in duplicate. Precision control charts will be maintained for each analyst and updated on a monthly basis by analyzing a minimum of twenty (20) replicates from a known positive sample. In addition, ten percent of all microbial analyses conducted in a participating laboratory will be verified by a second analyst.

4.5.3 Verification Checks

A minimum of ten (10) percent of all plate counts will be verified by a second analyst.

4.5.4 Confirmation Checks

Refer to **Section 9**, Microbial Methods for the Water Recovery Test, for confirmation tests which are specific for each microbiological method.

4.5.5 Sterility Checks

Sterility checks must be performed with each sample batch analyzed for enumeration. In addition, all media and reagent batches must be verified and documented for sterility.

Table 4-3 METHODOLOGY FOR ANALYSIS OF MICROBIOLOGY PARAMETERS[j]

PARAMETER	METHOD[l]	DESCRIPTION[k]	UNITS	DET'N LIMIT
Heterotrophic plate count[a]	Section-2.1.1	R2A, 28 C, 7days[b]	CFU/100 ML	1
Heterotrophic plate count[a]	Section-2.1.2	PCA, 28 C, 7days[b]	CFU/100mL	1
Aero-tolerant Eutrophic Mesophiles	Section-2.1.3	CAE[c], 35 C, 48 hr	CFU/100 ML	1
Fecal coliforms	Section-2.1.9	mFC, 44.5 C,24 hr	CFU/100 ML	1
Yeast and Mold	Section-2.1.6	Emmon's[d] 20 C, 5days	CFU/100 ML	1
Total Count	Section-2.2	epifluorescence	Cells/100 ML	approx.105
Gram positive	Section-2.1.5	PCA[e], 28 C, 7 days	CFU/100 ML	1
Gram negative	Section-2.1.4	PCA[f], 28 C, 7 days	CFU/100 ML	1
Anaerobes[g] (enumeration)	Section-2.1.10	Brewers Anaerobic Agar 35 C, 7 day	CFU/100 ML	1
Anaerobes (qualitative)	Section-2.1.11	Fluid Thioglycollate Medium, 35 C, 4 days	NA	NA
		Brewer Anaerobic Agar 35 C, 7 days	CFU/100 ML	NA
Enteric	Section-2.1.8	PCA[h], 35 C, 48 hr	CFU/100 ML	1
Legionellae	Section-2.1.7	BCYE[i], 35 C,10 day	CFU/100ML	333

a Includes culturable aerobes and facultative anaerobes

b Incubation times may be extended up to 21 days

c Chocolate Agar enriched with X and V factors

d Sabouraud Dextrose Agar with Rose Bengal and Chloramphenicol

Table 4-3 METHODOLOGY FOR ANALYSIS OF MICROBIOLOGY PARAMETERS[j] (continued)

[e] Plate Count Agar with phenylethanol

[f] Plate Count Agar with Crystal Violet

[g] Includes culturable obligate and facultative anaerobes

[h] Plate Count Agar with Bile Salts

[i] Supplemented with glycine, cysteine and antibiotics

[j] Appendix: Microbiological Methods For The Water Recovery Systems Test

[k] Maximum incubation intervals are listed

4.5.6 Analytical Control Samples

Currently few analytical control samples exist for microbiological parameters. **Table 4-4** lists the parameters for microbiological control samples planned for use as qualification samples during the Phase III activities. The qualification samples will be used as outlined in **Sections 3.3**. In addition, blind duplicate and sterile water samples may be submitted to the participating laboratories for microbiological analysis **(see Appendix 6)**. These may be sent to a single laboratory or split between two or more laboratories for analysis. In addition, each participating laboratory will be expected to participate in an approved interlaboratory control program such as the EPA laboratory monitoring program conducted by the Quality Assurance Branch, Environmental Monitoring Systems Laboratory located at Cincinnati, Ohio.

Table 4-4 TYPICAL MICROBIOLOGICAL PARAMETERS FOR QUALIFICATION, VERIFICATION, AND REFERENCE CHECK SAMPLES

Parameter	Concentration	Notes
Fecal coliforms	10^6 - 10^9	see note a
Gram Negatives	10^6 - 10^9	see note b
Gram Positives	10^6 - 10^9	see note b
Heterotrophs	10^6 - 10^9	see note b
Identification	N/A	see note c

Notes:

a includes 0-2 fecal coliforms for detection and/or enumeration

b includes Gram positive or Gram negative organisms for detection and enumeration

c includes 1 ATCC culture for isolation and identification

5.0 REQUIREMENTS FOR THE CHEMICAL ANALYSIS OF AIR

5.1 Sample Collection

Variables associated with field collection of air samples often affect the results more than the analytical procedures being used. Field sampling personnel should be familiar with sampling and measurement procedures and equipment to be used.

5.1.1 Physical Properties

The physical properties of the contaminant(s) being sampled are a major factor in determining the collection procedure to be employed. Physical properties which are important include boiling point, vapor pressure, polarity, and solubility in water and organic solvents. Other factors affecting the collection of airborne contaminants include temperature, humidity, sampling flow rates, chemical properties, volatility and concentration of the contaminant(s). Each of these must be considered when developing a sampling method and strategy.

5.1.2 Volatility

Organic compounds may be classified based on their degree of volatility. Each of these classes are briefly described below.

5.1.2.1 Volatile Organic Compounds

Volatile organic compounds (VOCs) may be characterized as non-methane organic compounds having vapor pressures greater than 10^{-2} kPa. These compounds predominately occur as gasses at standard temperature and pressure and may be collected and concentrated by high volume sampling on solid sorbents. The compounds are then thermally desorbed from the sorbent medium for analysis.

5.1.2.2 Semi-Volatile Organic Compounds

Semi-volatile organic compounds (SVOCs) represent a wide variety of organic contaminants which have vapor pressures which range from 10^{-2} to 10^{-8} kPa. SVOCs are present in air as gases and as condensed particle-bound constituents. Because of this sampling regimes for SVOCs must include distribution analysis for quantitative recovery.

5.1.2.3 Non-Volatile Compounds

Non-volatile organic compounds (NVOs) may be described as those compounds having vapor pressures less than 10^{-8} kPa. NVOs associated with ambient air are primarily recovered as condensed particle-bound constituents. The collection and concentration of NVOs from ambient air is usually accomplished by high volume sampling through particulate filters and subsequent extraction of the NVOs from the filter material.

5.1.3 Sampling Media

Various sampling media may be used for the collection and concentration of airborne organic contaminants. The sampling media will be specified for the parameter of interest by the method to be used. This may include specific filter types and porosity, concentration and volume of liquid media or amount, and type of solid sorbent to be used. Most sampling media commonly used are well characterized. If specific products are specified no substitutions will be made without the prior written approval by the PI, Medical Monitor, and/or NASA/MSFC Material and Processing Laboratory.

5.1.4 Sampling Equipment

Sampling equipment must be operated properly and maintained in good working condition. Periodic maintenance and inspection is a necessary function of the sampling personnel. Pumps should be chosen which are compatible with the sampling requirements outlined in a particular method. The pump must be capable of maintaining the recommended flow rates and sampling times required to meet the specified detection limits. All pumps must be calibrated prior to use. When practical, flow rates are measured during sample collection as well.

5.1.5 Record-keeping

Accurate record-keeping is essential. All pertinent information including sample location, calibration information, sampling times, sampler, temperature, humidity, possible interfering compounds, and any anomalies should be documented. The exact sampling time and flow rate are necessary to correctly estimate the volume of air sampled. This is accomplished using the initial calibration data, start and stop times and periodic spot checks to assure the pump is operating properly during the collection procedure.

5.1.6 Contamination

Since modern analytical techniques are extremely sensitive, special care must be taken to minimize contamination of field samples. Samples must not be stored or shipped with bulk materials which can contaminate the sample. Glassware or other containers used in sampling and/or shipping should be cleaned and free of any contaminating materials. Field blanks are routinely used to estimate the contamination which may occur during sampling, transport, and storage prior to analysis.

5.1.7 Sampling Schedule

A written sampling schedule will be developed and approved by the PI prior to sample collection. The schedule should include a statement of purpose, description of the area to be sampled, number and location of sample sites, collection and analysis methods to be used, number and type of samples and blanks to be collected and analyzed, and specific analytical controls to be employed.

5.2 Analytical Methods

Analytical methods to be used will be chosen from recognized sources. The primary source for the determination and quantification of organic contaminants from ambient air is the USEPA "Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air".[9] Secondary methods, if required, will be from the NIOSH "Manual of Analytical Methods" and/or APHA "Methods of Air Sampling and Analysis".[10,11] Specific parameters of interest and approved methods are listed in **Table 5-1**.

Table 5-1 RECOMMENDED METHODS FOR THE ANALYSIS OF SELECTED TOXIC ORGANIC CONTAMINANTS IN AMBIENT AIR[9,10]

COMPOUND	APPLICABLE METHODS
Acetaldehyde	TO-5
Acrolein	TO-5
Acrylonitrile	TO-2,TO-3
Allyl Chloride	TO-2,TO-3
Aldrin	TO-4,TO-10
Benzaldehyde	TO-5
Benzene	TO-1,TO-2,TO-3,TO-14
Benzo (a) Pyrene	TO-13
Benzylchloride	TO-1,TO-3,TO-14
Bromoform (Tribromomethane)	TO-1,TO-3
Bromobenzene	TO-1
Bromomethane	TO-14
Carbon Tetrachloride	TO-2,TO-3,TO-14
Chlordane	TO-10
Chlorobenzene	TO-1,TO-3,TO-14
Chloroethane	TO-14
Chloromethane	TO-14
Chloroform	TO-1,TO-2,TO-3
Cresols (o, m, p - Methyl Phenols)	TO-8
Cumene	TO-1
2,4,-D esters	TO-10
4,4'-DDE	TO-4,TO-10
4,4'-DDT	TO-4,TO-10
1,2-Dichlorobenzene	TO-14
1,3-Dichlorobenzene	TO-14
1,4-Dichlorobenzene	TO-1,TO-3,TO-14
Dichlorodifluoromethane	TO-14
1,2-Dichloroethane	TO-1,TO-2,To-3
1,1-Dichloroethene	TO-2,TO-3,TO-14
cis-1,2-Dichloroethylene	TO-14
1,2-Dichloropropane	TO-1,TO-14
1,3-Dichloropropane	TO-1
cis-1,3-Dichloropropene	TO-14
trans-1,3-Dichloropropene	TO-14
1,2-Dichloro-1,1,2,2-tetrafluoroethane	TO-14
Dieldrin	TO-10
Endrin	TO-10
Endrin Aldehyde	TO-10

Table 5-1 (continued) RECOMMENDED METHODS FOR THE ANALYSIS OF SELECTED TOXIC ORGANIC CONTAMINANTS IN AMBIENT AIR[9,10]

COMPOUND	APPLICABLE METHODS
Ethylbenzene	TO-1,TO-14
Ethylene Dibromide	TO-1,TO-14
4-Ethyltoluene	TO-14
Formaldehyde	TO-5,TO-11
Heptachlor	TO-10
Heptachlor epoxide	TO-10
1,2,3,4,7,8-Hexachlorodibenzo-p-Dioxin	TO-9
Hexachlorobenzene	TO-10
Hexachlorobutadiene	TO-14
B-Hexachlorocyclohexanes	TO-10
Lindane	TO-10
Methoxychlor	TO-10
Methylene Chloride	TO-2,TO-3, TO-14
Nitrobenzene	TO-1,TO-3
N-Nitrosodimethylamine	TO-7
Octachlorodibenzo-p-Dioxin	TO-9
Oxychlordane	TO-10
Pentachlorobenzene	TO-10
Pentachlorophenol	TO-10
Phenol	TO-8
Polychlorinated Biphenyls	TO-4
Propanal	TO-5
Styrene	TO-14
1,2,3,4-Tetrachlorodibenzo-p-Dioxin	TO-9
2,3,7,8-Tetrachlorodibenzo-p-Dioxin	TO-9
1,1,2,2,-Tetrachloroethane	TO-14
Tetrachloroethylene	TO-1,TO-3,TO-14
Toluene	TO-1,TO-2,TO-3,TO-14
1,2,4-Trichlorobenzene	TO-14
1,1,1-Trichloroethane	TO-2,TO-3,TO-14
1,1,2-Trichloroethane	TO-14

Table 5-1 (continued) RECOMMENDED METHODS FOR THE ANALYSIS OF SELECTED TOXIC ORGANIC CONTAMINANTS IN AMBIENT AIR[9,10]

COMPOUND	APPLICABLE METHODS
Trichlorofluoromethane	TO-14
1,1,2-Trichloro-1,2,2-trifluoroethane	TO-14
2,4,5-Trichlorophenol	TO-10
1,2,4-Trimethylbenzene	TO-14
1,3,5-Trimethylbenzene	TO-14
Vinyl Chloride	TO-2,TO-3,TO-14
o, m, p-Xylene	TO-1,TO-3,TO-14

5.3 Analytical Control Procedures / Air

Analytical control procedures for air sampling and anayses are identical to those utilized for water. Special care should be taken to ensure that "dead leg" volumes are not collected during sampling. In addition, special care should be taken during subsystem gas sampling, to ensure that leaks are not present. Air samples should be stored at room temperature until analyzed.

6.0 REQUIREMENTS FOR MATERIALS AND PROCESS CONTROLS

6.1 Sample Collection and Preparation

Samples will be collected by Boeing Environmental Laboratory personnel according to the daily sampling schedule created by the MSFC Test Laboratory (EL65) data custodian, and in conjunction with the batch designations developed by the ACC. The collected samples will be transferred to the laboratory area where batch and sample numbers will be assigned, appropriate control samples added and the samples recorded into the Data Log. All bottles will be prepared and delivered to the EL65 data custodian at least 12 hours prior to sampling for these samples to be sent by hired courier. Control samples will be delivered the day of the test. **Figure 6-1** illustrates the logistics of sample collection activities.

6.2 Field Numbers

At the time of collection, samples will be logged into a Field Notebook and assigned a field number. The field notebook will remain with the samplers. Field numbers will be assigned as follows:

B-XX-AA-BB-CCC

Where,

B = The Boeing Laboratory

XX = A sequentially numbered field log book

AA = The page on which the sample is recorded

BB = The entry of the sample on page AA

CCC = The initials of the sampler

The field number must be included on the sample label and chain-of-custody form for tracking purposes.

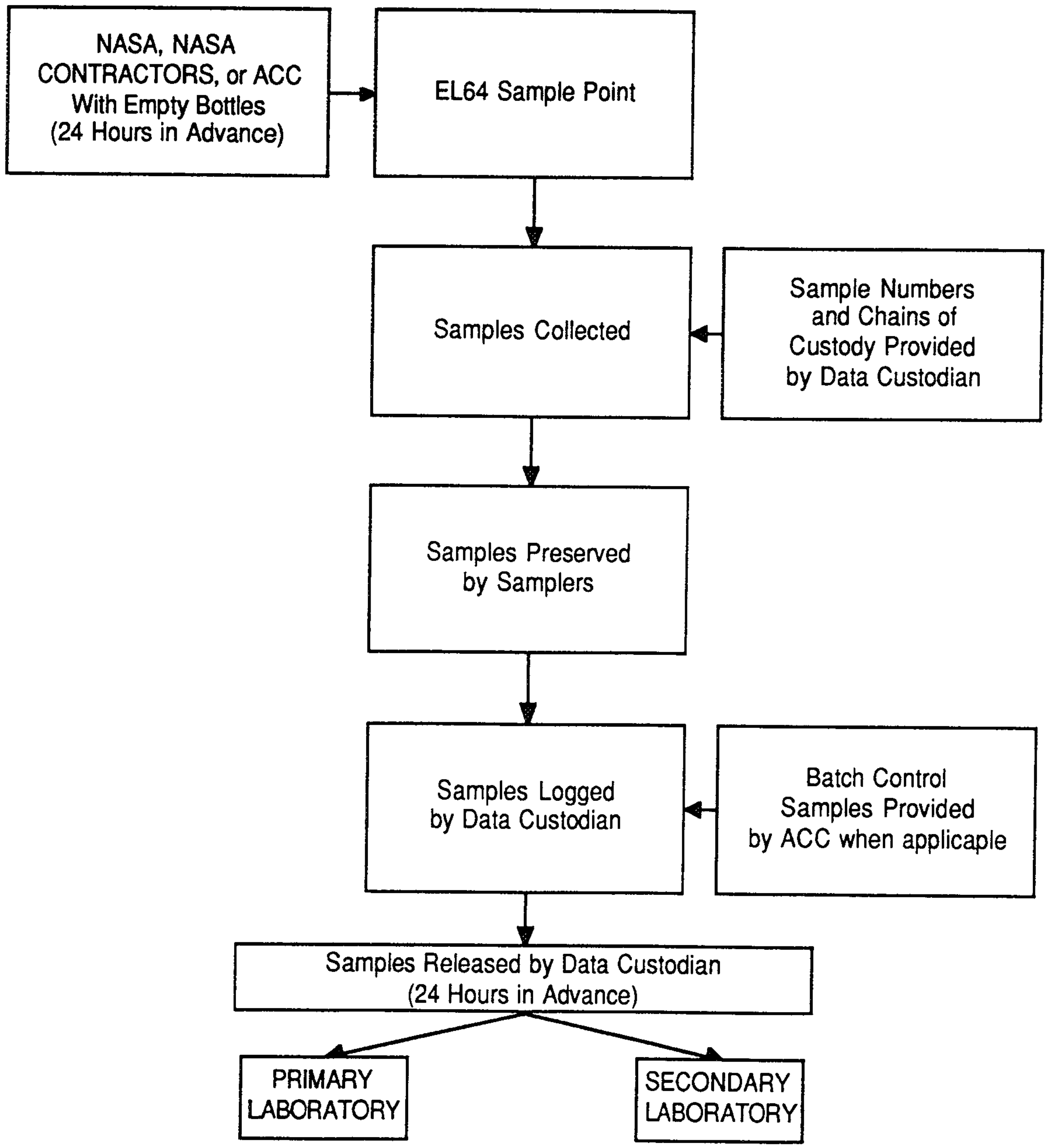

Figure 6-1 SAMPLING COLLECTION AND LOGISTICS FLOW CHART

6.3 Sample Labels

Sample collection labels will be prepared and affixed to each sample container. The sample labels should minimally contain the following information:

- o NASA Sample collection number
- o Date/Time collected
- o Collection location/description
- o Initials of personnel collecting the sample
- o Parameters for analysis
- o How preserved
- o Any anomalies encountered during sampling
- o Laboratory to which the sample is to be sent
- o Field tracking number

An illustration of the sample label is provided in **Figure 6-2**.

6.4 Sample Log In

Immediately after collection, samples will be transferred to the Laboratory located in building 4755. At this point samples will be logged into a Master Log Book and assigned a permanent sample number. The log book will be maintained by the Lead Sampler and will remain on-site at the Test Facility located at MSFC Building 4755. In addition, samples will be logged by the Data Custodian into the electronic Data Log. During computer down time the Data Log will be kept in a bound log book. The sample numbers will consist of the test stage, test day, batch assignment, sample group and subgroup, sample port and laboratory assignment (27 spaces total).

Example: XXX-AAA-BB-C-DDDD-EEE-F-GGG

Where,

-XXX	The test name (WRT)
-AAA	The test Stage (1A, 3A, etc.)
-BB	The Test Day (1-99)
-C	The particular batch (1-4)
-DDDD	The analysis group and subgroup
-EEE	The sample port (1-999)
-F	The laboratory assigned to conduct the analysis
-GGG	The unit/tank description

SAMPLE NUMBER
Date/Time:

Sampler Name:

Field Tracking #:

Preservative:

Remarks:

Required
Volume:

Parameters for Analysis:

Figure 6-2 ILLUSTRATION OF SAMPLE LABEL

In addition, the following information should be included in the Sample Log Book:

- Date/Time Collected
- Collected By (initials)
- Sample Description
- Analysis Group and Subgroup
- Preservative
- Comments
- Field Number (if applicable)

An example of the layout for the Sample Log is illustrated in **Figure 6-3** (This example follows the old sample number scheme of 25 spaces).

ANY SIMILAR CONFIGURATION MAY BE USED WITHOUT ACC APPROVAL AS LONG AS THE INFORMATION BELOW IS RECORDED AS A MINIMUM.

Report Date	Test:	Stage:	Day:

Sample Number	Date/Time Collected	Field Number	Description	Parameters For Analysis

Figure 6-3 LAYOUT OF SAMPLE LOG

6.5 Electronic Data Entry

Electronic data entry will be used as an alternative to the manual log in procedure. When using electronic data entry data should be saved after every ten (10) entries. Additionally, duplicate backup of the Data Log data should be made following each data entry session. One copy will be maintained at the test site at all times and the second copy will be in the custody of the Data Custodian.

6.6 Chain of Custody

At the time samples are recorded into the sample log book a chain of custody form is also completed for each sample batch. At this time the samples are relinquished to the Data Custodian for distribution to the participating laboratories **(see Appendix 4)**. A copy of the chain of custody form is illustrated in **Figure 6-4**.

6.7 Sample Storage

Samples will be segregated by batch and the participating laboratory to which the samples are to be sent. Samples will be stored at 4°C until relinquished by the Data Custodian.

6.8 Analysis of Test Parameters

Test parameters will be analyzed simultaneously with regular test samples within the assigned batches. Results will then be summarized per individual samples. Analysis data will be reviewed by the ACC and compared to internal batch controls and parameters labeled as "suspect" or "acceptable". It should be understood that "Suspect" data does not place blame with any particular group. When data is marked "Suspect", this only indicates that the QC associated with the parameter has identified a problem. The problem may lie with the participating laboratory, the ACC, and/or the control sample itself. For this reason, "Suspect" data should be treated with scrutiny but blame for the problem should not be directed to any one individual or organization. By treating data in this way, all parties involved in WRT work will be ensured that the data generated is of the highest possible quality.

Such review will be carried out through the utilization of AC reports generated by the FEDS. When the FEDS is not operable, a manual method of hard-copy review will be utilized. Hard copy data should follow a scheme **similar** to that depicted in **Figure 6-5**. Reports of "Acceptable" ("A") and "Suspect" ("S") ratings will be available for review at the request of NASA/MSFC ED62. "S" data may be evaluated by a review committee headed by the NASA/MSFC Materials and Process Laboratory for final disposition.

6.9 Analysis of Control Samples

Results obtained from intralaboratory control samples should be transmitted to the FEDS and faxed directly to the ACC. This data will be used by the ACC and/or review committee to make a final decision regarding the validity and/or limitations of "suspect" data.

BOEING LABORATORIES CHAIN OF CUSTODY RECORD

TYPE OF TEST:	SUBSYSTEM & LOCATION SAMPLED:	ANALYSIS REQUIRED
CUSTOMER:	CONTACT:	
BATCH # ______	PROTOCOL GROUP: ______ PARAMETER GROUP ______	

SAMPLE NO	SAMPLE DATE	SAMPLE TIME	SAMPLE *G	SAMPLE *W	FIELD TRACKING NUMBER	FIELD TRACKING NUMBER	NO. OF CONTAINERS	ANALYSIS REQUIRED	COMMENTS

1 SAMPLED BY & TITLE (SIGNATURE)	DATE/TIME	2 RELINQUISHED BY (SIGNATURE)	DATE/TIME	3 RECEIVED BY (SIGNATURE)	DATE/TIME		
4 RELINQUISHED BY (SIGNATURE)	DATE/TIME	5 RECEIVED BY (SIGNATURE)	DATE/TIME	6 RELINQUISHED BY (Signature)	DATE/TIME	7 RECEIVED BY	DATE/TIME
8 RELINQUISHED BY (SIGNATURE)	DATE/TIME	9 RECEIVED BY MOBILE LAB FOR FIELD ANALYSIS	DATE/TIME	10 DISPATCHED BY	DATE/TIME	11 RECEIVED FOR LAB BY	DATE/TIME

ion

Figure 6-4 CHAIN OF CUSTODY

ORGANICS

NASA TRACKING NUMBER	PARAMETER	RESULT	UNITS	COMMENTS

SURROGATES:

RECOVERIES:

SPECIAL NOTES:

INORGANICS

NASA TRACKING NUMBER	PARAMETER	RESULT	UNITS	COMMENTS

SPECIAL NOTES:

Figure 6-5 LABORATORY HARD COPY REPORTS

MICROBIOLOGICAL

NASA TRACKING NUMBER	PARAMETER	RESULT	UNITS	COMMENTS

COLLECTION TIME:

MICRO DILUTION START TIME:

SPECIAL NOTES:

Figure 6-5 LABORATORY HARD COPY REPORTS
(continued)

6.10 Intralaboratory Data Handling and Reporting

The intralaboratory documentation system for data handling and reporting will be described. This should include all documentation and associated procedures for sample tracking. In addition, the flow process procedures for completion of data sheets for intralaboratory use should be defined and illustrated. Responsible personnel should be identified for each phase of the documentation system.

Report formats for collection and interpretation of unknown and control data should be described. Reported data should only contain significant figures. The following are guidelines[1] for determining significant figures:

(1) A value is made up of significant figures when it contains all digits known to be true and the last digit in doubt. For example, if a figure is reported as 18.8 mg/L, the 18 must be firm while the 0.8 is somewhat uncertain, but presumably better than one of the values 0.7 or 0.9.

(2) Final zeros after a decimal point are always meant to be significant figures. For example, to the nearest milligram, 9.8 is reported as 9.800 g.

(3) Zeros before a decimal point with nonzero digits preceding them are significant. With no preceding nonzero digit, a zero before the decimal point is not significant.

(4) If there are no nonzero digits preceding a decimal point, the zeros after the decimal point but preceding other nonzero digits are not significant. These zeros only indicate the position of the decimal point.

Proper use of significant figures provides an indication of the reliability of the analytical method used. Analysts should take care not to report a result with more digits than are significant.

The following rules[1] for rounding numbers will apply to all reported data:

(1) Rounding off of numbers is a necessary operation in all analytical areas. It is automatically applied by the limits of measurement of every instrument and all glassware. However, when it is applied in chemical calculations incorrectly or prematurely, it can adversely affect the final results.

(2) If the figure following those to be retained is less than 5, the figure is dropped, and the retained figures are kept unchanged. As an example, 11.443 is rounded off to 11.44.

(3) If the figure following those to be retained is greater than 5, the figure is dropped, and the retained figure is raised by one. As an example, 11.446 is rounded off to 11.45.

(4) If the figure following those to be retained is 5, and if there are no figures other than zeros beyond the five, the figure 5 is dropped, and the last-place figure retained is increased by one.

(5) When a series of numbers is added, the sum should be rounded off to the same number of decimal places as the addend with the smallest number of places. However, the operation is completed with all decimal places intact, and the rounding off is done afterward.

(6) When one number is subtracted from another, rounding off should be completed after the subtraction operation, to avoid possible invalidation of the operation.

(7) When two numbers are to be multiplied, all digits are carried through the operation, then the product is rounded off to the number of significant digits of the multiplier with the fewer significant digits.

(8) When two numbers are to be divided, the division is carried out on the two numbers using all digits. Then the quotient is rounded off to the number of significant digits of the divisor or dividend, whichever has the fewer.

(9) When a number contains n significant digits, its root can be relied on for n digits, but its power can rarely be relied on for n digits.

The preceding rules for rounding off are reasonable for most calculations; however, when dealing with two nearly equal numbers, there is a danger of loss of significance when applied to a series of computations that rely on a relatively small difference in two values. Examples are calculation of variance and standard deviation. The recommended procedure is to carry several extra figures through the calculations and then to round off the final answer to the proper number of significant figures.

6.11 Interlaboratory Data Handling and Reporting

Laboratory results will be reported to the ACC through the FEDS, or through laboratory hard copy data. Similar data sheets will be forwarded to the Principal Investigator and the Test Conductor by the Data Custodian. The Data Custodian will immediately forward a copy of all results obtained from fast turnaround samples to the NASA/MSFC WRT Medical Monitor **(see Appendix 7)**. **Figure 6-6** illustrates the flow of data from the participating laboratories. **Figure 6-7** and **6-8** illustrate the flow of information pertaining to suspected pathogens.

6.12 Additional Test Requests

During the course of sample analysis, the chemical or microbial analyst may inadvertently come across parameters that should be analyzed. In order to make a place for such data in the FEDS, the Additional Test Request Form is provided to the participating laboratories. The form is filled out by the analyst and submitted to the Data Custodian for approval and datalog entry. A sample number will be provided by the Data Custodian to the laboratory for the sample in question **(Figure 6-9)**.

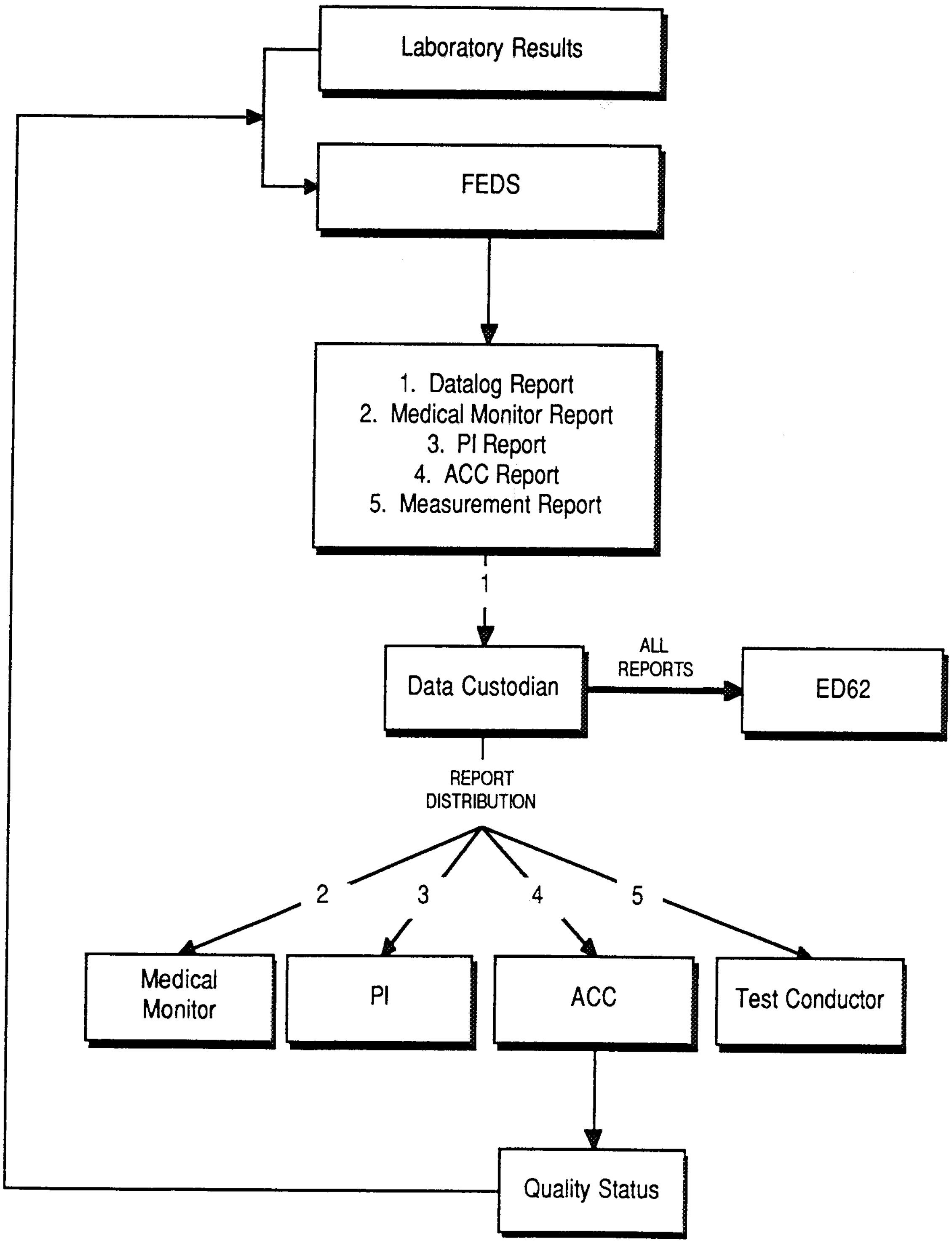

Figure 6-6 REPORT FLOW CHART

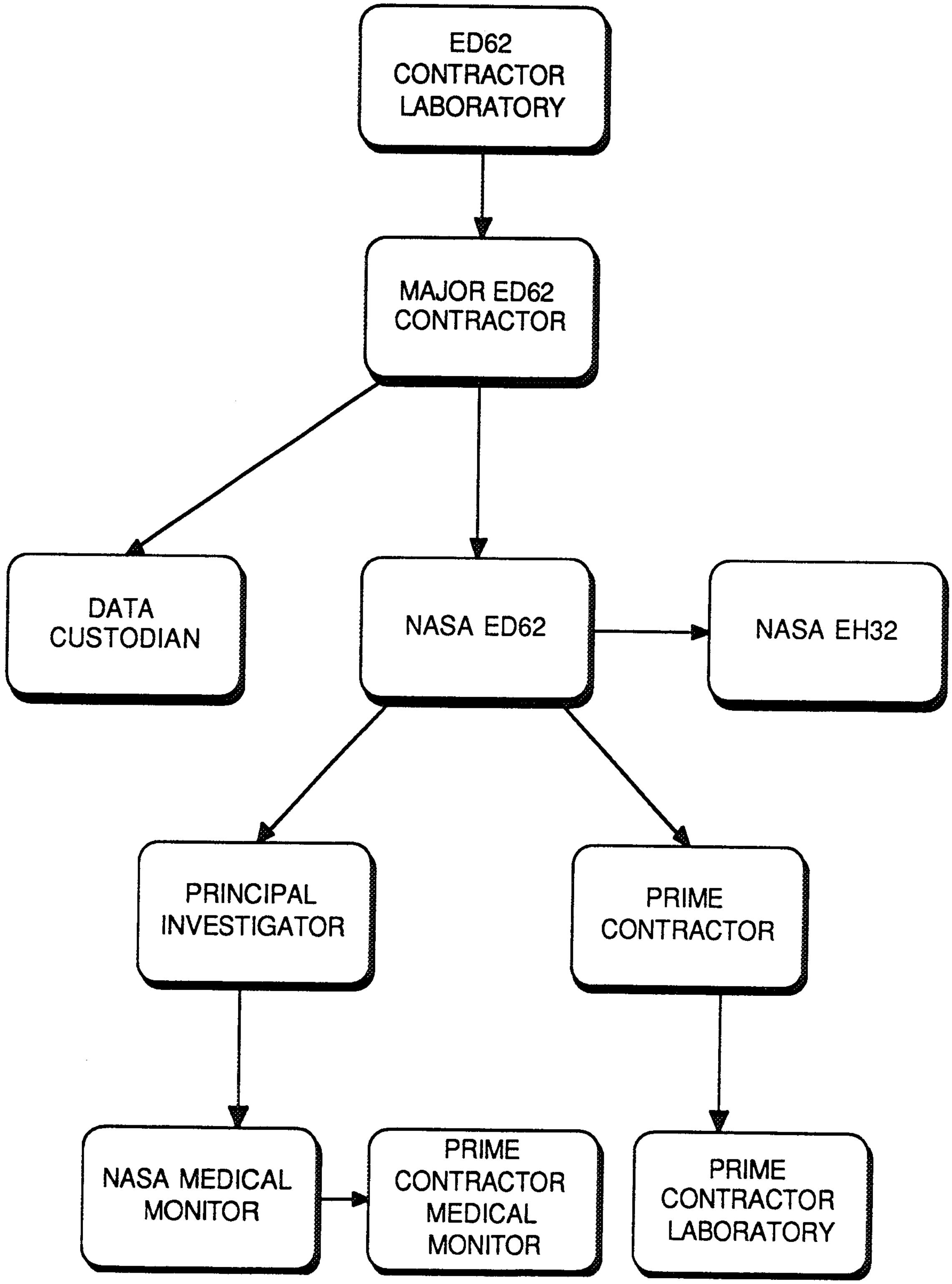

Figure 6-7 CHAIN OF COMMUNICATION
ED62 MAJOR CONTRACTOR LABORATORIES
PRESUMPTIVE PATHOGENS

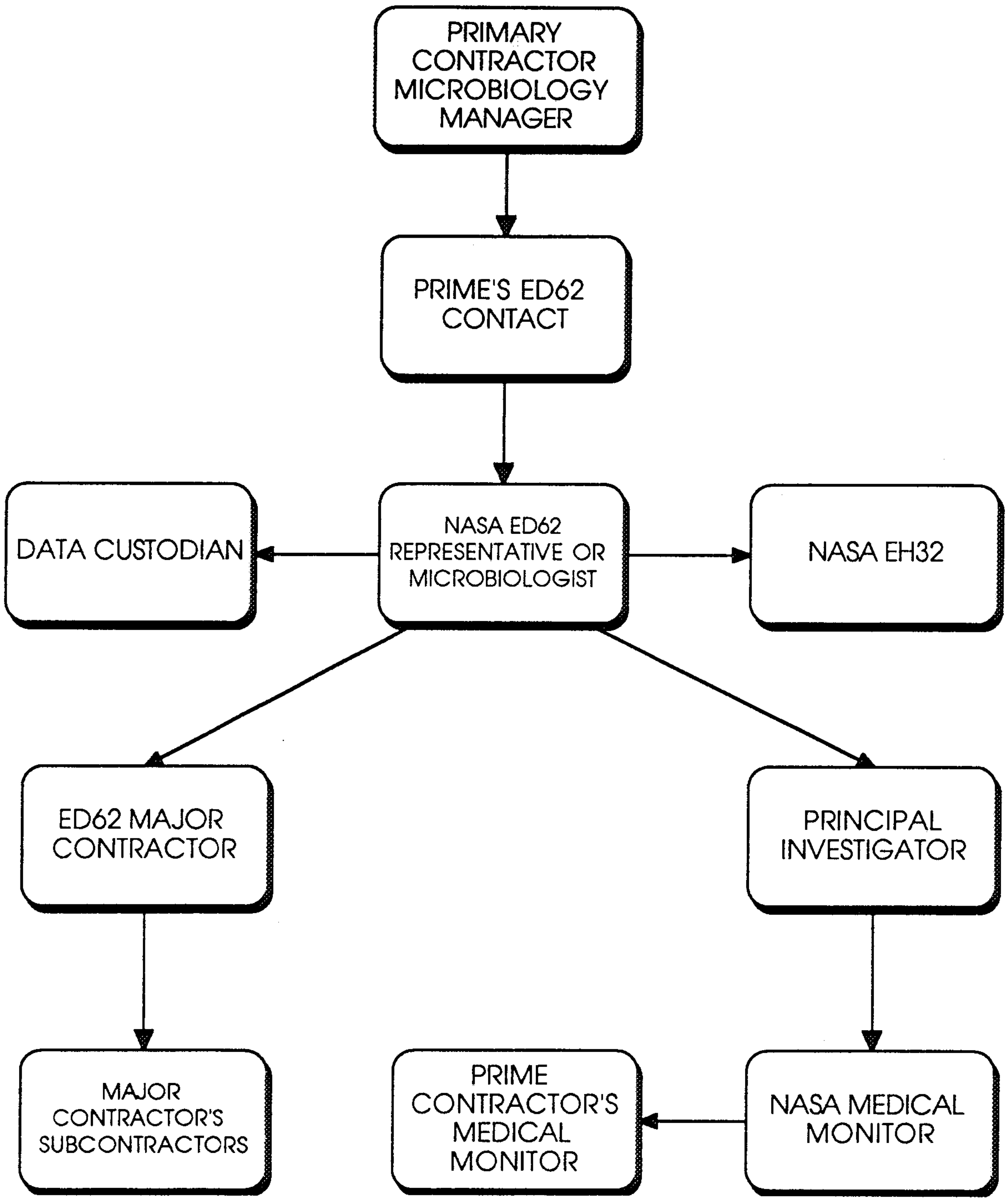

Figure 6-8 CHAIN OF COMMUNICATION
PRIMARY CONTRACTOR PRESUMPTIVE PATHOGENS

ADDITIONAL TEST REQUEST

ED62 CONTRACTOR SAMPLE NUMBER: ____________________

NASA SAMPLE NUMBER: ____________________

FIELD TRACKING NUMBER: ____________________

DATE REQUESTED: ____________________

TESTS REQUESTED: ____________________

____

____

____

____

____

____

REQUESTED BY: ____________________

RECEIVED BY (DATA CUSTODIAN): ____________________

DATE: ____________________

ADDITIONAL NASA SAMPLE NUMBER(S) ASSIGNED: ____________________

____

Figure 6-9 SAMPLE ADDITIONAL TEST REQUEST FORM

6.13 Control Sample Protest Procedure

Occasionally, it is necessary for the ACC to reevaluate control data following the rating of laboratory data. These evaluations are initiated as protests by a laboratory participating in the WRT, and are based on the lab's in-house data and experience with other analyses for the parameter in question. Protests of data ratings (Suspect vs Acceptable; i.e. "S" vs "A") are handled according to the steps described in "Protest Procedure" below.

6.13.1 Suspect Data

It should be understood that "Suspect" data does not place blame with any particular group. When data is marked "Suspect", this indicates that the QC associated with the parameter has identified a problem. The problem may lie with the participating laboratory, the ACC, and/or the control sample itself. For this reason, "Suspect" data should be treated with scrutiny, but blame for the problem should not be directed to any one individual or organization. By treating data in this way, all parties involved in WRT work will be ensured that the data generated is of the highest possible quality.

6.13.2 Protest Procedure

Step I

A written protest of a rating for a parameter and a particular sample is delivered to the ACC by the participating laboratory. The protest must include, as a minimum, the NASA sample number, the parameter in question (including its parameter type as defined by the NASA database), raw analytical data (including any curves and/or chromatogram etc.), the calibration data pertaining to the sample in question, and a short explanation of the protest.

Step II

The ACC reviews the protest document, and makes a decision as to the acceptability of the protest. Should the ACC determine a protest to be non-acceptable or unresolvable, a written explanation will be delivered to the participating laboratory within ten working days of the protest. Unresolvable protests include those for which there is prior evidence of acceptable performance on the protested lot, and no physical evidence of contamination caused by the ACC, or the vendor laboratory. The ACC performs his duties for NASA with the integrity of the WRT as a primary concern. For this reason, all ACC decisions regarding QA/QC and the WRT must be final. When protests are determined, at this step, to be unresolvable, a report of the protest will be submitted by the ACC to NASA. The report will include a brief explanation of the ACC's decision, and a brief memo from the participating laboratory explaining the reason for the protest.

Step III

Should the ACC choose to accept the protest as potentially valid, steps will be taken to collect evidence for and against the claim of the protest. Two samples are submitted to the protesting laboratory for analysis. These samples are submitted only between WRT stages and thus will not impact scheduled work. One or both of the samples submitted may contain the parameter in question, and one sample may be from the protested lot (based on the availability of the lot in question).

The samples are analyzed by, and at the expense of, the protesting laboratory. They are used to demonstrate an ability, on the part of the protesting laboratory, to provide an accurate analysis for a specific parameter. The protesting laboratory will have the option of analyzing the samples during down time, or to wait for further NASA testing. If the samples are requested during regular testing, they will be delivered along with the usual control samples. All sample results must be returned to the ACC within 20 working days of the date the samples were delivered to the protesting facility. Results received by the ACC following the deadline will not be considered for review.

Should the result reported, for the protested parameter (on a check sample from a lot other than the protested lot), be out of the acceptable range, the protest is regarded as invalid. For all invalid protests, no further action is taken. There will be no opportunity for further protest on the same sample. Should the results reported for the check sample be within the acceptable range, the protest is rated as valid (see "Valid Protest"), unless the analysis of the protested lot is also analyzed within the acceptable range in which case the protest is "Invalid" (Please refer to the table below, and the chart at the end of this section for a complete listing of possible rating combinations). These and other factors are summarized below. The ratings in the table (acceptable vs suspect) are the ratings received for the samples re-submitted to the participating laboratory (either Lot 1, Lot 2, or the Original Lot).

CASE	NEW LOT 1	NEW LOT 2	ORIGINAL LOT	PROTEST
1.	Acceptable	Acceptable	------------	Valid
2.	Acceptable	----------	Acceptable	Invalid
3.	Acceptable	----------	Unacceptable	Valid
4.	Unacceptable	Unacceptable	------------	Invalid
5.	Unacceptable	Acceptable	------------	Invalid
6.	Acceptable	Unacceptable	------------	Invalid
7.	Unacceptable	-----------	Acceptable	Invalid
8.	Unacceptable	----------	Unacceptable	Invalid

Note: It is possible to receive two "Acceptable" ratings for the check samples, but an "Invalid" rating for the protest (i.e. If a re-submitted sample from a protested lot is analyzed within the acceptable limits on the second try, then the problem must be assumed to be from a source other than the QC sample lot, and an "S" flag will result (i.e. Invalid Protest).

Valid Protest-

The procedure for corrective action is as follows:

The ACC makes note of the corrupt parameters, and removes them from further use within the lot in question. In addition, the QC parameter for the sample in question is removed from the AC table in the database, and the previously assigned AC rating is changed to an "N" for "Not Controlled". Any reports generated prior to the protest which contain the invalidated rating(s) are updated. These updates consist of an addendum to the report from the ACC. Each addendum is delivered to both NASA and the participating laboratory.

Invalid Protest-

Invalid protests require only a written explanation of the decision by the ACC to the protesting laboratory. Inconsistent analyses for any particular lot must be treated as "Suspect" to uphold the integrity of the program (See **Paragraph 1, Subsection 6.13.1**, for a definition of the term "Suspect").

6.14 ACC Report Generation

Reports are generated by the ACC, as necessary, regarding the status of the program, the availability of data, and summaries of data flags (Acceptable vs. Suspect). These reports are generated for NASA and all groups involved with the WRT and data evaluation. Before reports are generated, the ACC will make certain that any "Suspect" data is reviewed by the participating laboratory producing the flagged result. This system will assist the ACC in providing a complete report with little need for an addendum. In addition, the procedure will ensure that high quality data, reviewed by both the ACC and the participating laboratory, is available for evaluation in the FEDS. These reports will be generated based on data queried from FEDS. As a result, any delay in the trasmission of data from the laboratory to FEDS will result in a delay of the final ACC report for the stage in question.

7.0 SAFETY

Each participating laboratory should have a written Laboratory Safety document. As a minimum the Laboratory Safety document should address the following:

- o General Laboratory Guidelines
- o Fire and Emergency Procedures
- o Chemical Hazards
- o Biological Hazards
- o Radiological Hazards
- o Reporting of incidents and accidents
- o Emergency Medical Care
- o Safety Training
- o Log of personnel who has read the document

8.0 REFERENCES

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7. Winter, J., Britton, P., Clements, H. and Kroner, R., "Total Mercury in Water," EPA Method Study 8, USEPA, Office of Research and Development, EMSL, Cincinnati, OH 45268, 1977.

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9. Riggin, R. M. "Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air," EPA-600/4-84-041, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, 1984.

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MICROBIOLOGICAL METHODS
FOR THE
WATER RECOVERY TEST

9.0 MICROBIOLOGICAL METHODS FOR THE WATER RECOVERY SYSTEMS TEST

9.1 INTRODUCTION

Current microbiological parameters specified to verify microbiological quality of Space Station Freedom water quality include the enumeration of total bacteria, anaerobes, aerobes, yeasts and molds, enteric bacteria, gram positive, gram negatives, and Escherchia coli. In addition, other parameters have been identified as necessary to support the Water Recovery Test (WRT) activities. These include: aerotolerant eutrophic mesophiles, legionella, and an additional method for heterotrophic bacteria.

If interlaboratory data are to be compared to evaluate data quality, analytical methods must be eliminated as a variable. Therefore, each participating laboratory must utilize the same analytical methods and procedures. Without this standardization, data can be neither compared or validated between laboratories.

Multiple laboratory participation represents a conservative approach to insure quality and completeness of data. Invariably sample loss will occur in transport and analyses. Natural variance is a reality on any test of this magnitude and is further enhanced because biological entities, capable of growth and death, are specific parameters of interest. The large variation due to the participation of human test subjects has been noted with previous testing.

The purpose of this document is to provide standardized laboratory procedures for the enumeration of certain microorganisms in water and wastewater specific to the WRT. It is not intended, nor is it implied, to be a complete microbiological methods manual.

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9.2 ENUMERATION PROCEDURES

9.2.1 Membrane Filtration Method

Sample preservation and storage. Sodium thiosulfate at a concentration of 10% should be added to samples (0.1 mL $Na_2S_2O_3$/100 mL sample) where residual chlorine or other halogen is suspected.

Samples suspected of containing copper, zinc, or heavy metals should be additionally preserved using a solution of disodium salt of ethylenediaminetetraacetic acid (EDTA) as a chelating agent. 0.3 mL of a 15% solution of EDTA at concentrations higher than 0.01 mg/L, adjusted to pH 6.5, is used per 100 mL of sample.

Note: The sodium thiosulfate and EDTA solutions should be added to the sample collection bottles prior to autoclaving. These solutions may be combined and added as a single solution.

Samples not processed within one hour of collection should be stored between 4° and 10°C until analyzed except for qualitative and quantitative anaerobic samples which are stored at 25°C.

Note: Method 9.2.1.7 Enumeration of Legionella, and Method 9.3.2 Presence of anaerobic bacteria are an exception to the sample preservation procedure stated above. For applicable procedures see individual methods.

Media preparation. Media for this test is prepared by dispensing 5 mL of sterile molten media into 50 x 9 mm diameter sterile petri dishes. See appropriate method in the following sections for specific media preparation.

Sterilization of apparatus and materials. All glassware, filter units, filter holders, and utensils are presterilized at 121°C for 15 minutes.

Membrane filters. 47 mm diameter, 0.02 µm porosity, white gridded membranes (Millipore Corporation, Bedford, MA; catalog no. HAWG04751) are used for this procedure except where indicated otherwise by a specific method. These filters should be purchased presterilized.

Volume requirements. When less than 20 mL of sample (including diluent) is to be filtered, add approximately 10 mL of sterile buffered dilution water to the funnel before filtration. Unequal distribution of bacteria may occur if less water is filtered. Plates which lack uniform distribution cannot be counted.

The membrane filtration test has a range of limits for colonies per plate. Depending on the specific parameters of interest, acceptable countable ranges are 20-60 or 20-80 colonies per filter.

Dilution and Rinse Water. Sterile peptone dilution and rinse water will be used for all dilution and rinse procedures except where specifically noted. A 10% peptone water stock solution is prepared and autoclaved. The dilution/rinse water is prepared by diluting a measured volume of the sterile stock solution to a final concentration of 0.1%. The final pH is adjusted to 6.8 ±0.2 at 25°C. The dilution water is dispensed to provide 99 ±2 mL after autoclaving at 121°C for 15 minutes. The rinse water is dispensed in quantities convenient for handling (approximately 1 L). The prepared rinse water is autoclaved at 121°C for 20 minutes prior to storage and/or use. All peptone dilution and rinse water must be checked for sterility prior to use. Any bottles demonstrating turbidity should be discarded. If more than 10% of the bottles are contaminated the entire batch must be discarded. Record these results in the MPLB.

Note: Bacteria should not be suspended for more than 30 minutes.

Sample Dilution. To make a 1:100 dilution, transfer 1 mL of the water sample to 99 mL of the sterile dilution water blank (tube No. 1) and filter 1 mL after addition of the inoculum. Filter 10 mL from tube No. 1 to obtain a 1:10 dilution. For a 1:1000 dilution, transfer 1 mL from tube No.1 to a second 99 mL sterile dilution water blank (tube No. 2) and filter 1 mL.

Sample aliquots. Duplicate aliquots of 100 mL (or the maximum filterable volume), 10 and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01 and 0.001 mL are also prepared and filtered. The filters are then aseptically transferred to plates containing the appropriate medium.

Stressed or damaged bacteria. Samples containing urine pretreatment and/or brine mixtures should be neutralized using sterile phosphate buffer prior to membrane filtration. This will reduce the possible inhibition or damage of microorganisms on the filter surface due to the low pH and/or ionic concentration of these samples. Sterile phosphate buffer will be used to prepare sample aliquots and sample dilutions and to rinse the funnels (section 2.1). Sample aliquots of 10 and 1 mL are added directly to 99 mL of sterile phosphate buffer, shaken as recommended and the entire contents filtered. Sample dilutions of 0.1, 0.01, and 0.001 mL are prepared as described in the membrane filtration dilution method (section 2.1). Sample volumes exceeding 10 mL cannot be analyzed using this procedure.

Quality Control. Prepare at least two replicate plates for each sample aliquot and dilution used. Insert a sterile dilution water blank (99 mL) at the start of each sample analysis to check for contamination. Record the results on the worksheet.

Incubation. Incubate the plates inverted as directed in the procedure used.

Interpretation of results. Count the plates as directed in the appropriate procedure using a Quebec type colony counter. **Do not use electronic counting probes to count colonies since many of the isolates must be subsequently identified.** The results are then recorded on the Worksheet.

If counts from all membrane filters are zero, calculate the number of colonies per 100 mL that would have been reported if there had been one colony on the filter representing the largest filtration volume for that sample. Report as less than (<) that number of colonies per 100 mL.

If counts are less than the acceptable countable range (<20) but greater than zero, count the actual number of colonies noted on the plates representing the lowest dilution. Calculate the number of colonies per 100 mL and report this number.

If colonies are too numerous to count, use the upper limit count from the smallest filtration volume for that sample. Report as greater than (>) that number per 100 mL.

If there is no result because of confluency, lab accident, etc., report as "No Result" and specify reason.

Counts obtained from the sterile dilution blank must be less than or equal to 1 CFU per plate. If the number exceeds this limit the entire sample analysis is labeled as "suspect" and appropriate action is taken to identify and resolve the problem.

Successive filtrations. Decontaminate the funnels between successive filtrations by using an ultraviolet sterilizer. Irradiate 2 minutes with the funnel upside down, then invert and irradiate 3 minutes. Filtration equipment should be autoclaved between filtration series. A filtration series ends when 30 minutes elapse between successive filtrations or every ten (10) samples.

Note: Do not expose the medium or membrane filter culture preparations to random UV radiation leaks that might emanate from the sterilization cabinet. Eye protection is recommended.

Procedure

1) Aseptically place a sterile membrane filter over porous plate of receptacle. Carefully place the funnel unit over receptacle and lock it in place.

2) Shake sample bottle vigorously (approximately 25 times in 7 seconds) to evenly distribute the bacteria. Take care to secure the screw-cap and prevent leakage during shaking.

3) Filter sample under partial vacuum ($\leq$ 10 psi). Higher vacuum may result in cell damage/death and erroneous counts.

4) With filter still in place, rinse funnel by filtering three 20-30 mL portions of sterile peptone rinse water.

5) Unlock and remove the funnel, immediately remove membrane filter with sterile forceps, and place it on sterile pad or agar with a rolling motion to avoid entrapment of air.

9.2.1.1 Enumeration of Aerotolerant Heterotrophic Bacteria using R2A Agar

This procedure is designed for the isolation and enumeration of heterotrophic bacteria from water samples containing low concentrations of organic carbon. The medium used, R2A, is a low nutrient medium designed to culture heterotrophic bacteria. This includes oligotrophic bacteria characteristically found in these aquatic environments.

Procedure

Sample preservation and storage. See **Section 9.2.1**.

Preparation. Suspend 18.2 grams of Bacto R2A medium (Difco Laboratories, Detroit, MI) in 1 L of deionized or distilled water. The final pH of the medium should be 7.2 ± 0.2 at 25°C. If necessary adjust the pH using solid K_2HPO_4 or KH_2PO_4 prior to boiling.

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat.

Sterilization. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Dispensing. Within a LF hood, aseptically dispense approximately 5 mL of the sterile medium into sterile 50 x 9 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Quality Control. The prepared medium should be light amber in color and translucent. Darkened medium may indicate an increased time or temperature in sterilization and should not be used

Allow plates to incubate at room temperature, in the dark for 24 hours, and inspect for contamination prior to use. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Prepare a positive control using *Staphylococcus epidermidis* (ATCC 12228). The resultant colonies should appear small, round and smooth with a creamy white color. Record the results in the Media Preparation Log Book (MPLB). This is not a selective medium, therefore a negative control is not required.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane filters. See **Section 9.2.1**.

Sample application. Follow the Membrane Filtration Method described in Section 9.2.1. Duplicate aliquots of 100, 10, and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to the plates.

Incubation. Incubate the cultures aerobically at 28 ± 0.5°C for up to 7 days. Examine the plates after 24 and 48 hours and again after 7 days as some oligotrophic bacteria may require long incubation periods. Count the colonies at the time of each observation.

Interpretation of results. There may be a variety of colony morphologies and pigmentation as this procedure will culture many bacterial types. Count all colonies present. The countable range of colonies is between 20-80 colonies per plate. Plates should be counted using the magnifying lens of a Quebec type colony counter.

Confirmation. Different colonial morphologies will be Gram stained and their cellular morphology will be recorded. A minimum of two plates within the acceptable countable range (20 - 80 colonies/plate) should be chosen. A minimum of 10% of all colonies for each colonial morphology will be identified.

Note: All colony types will be characterized and/or identified based on the specific sample port location where isolated. For further clarification refer to NASA/MSFC document ED-62 (58-90).

9.2.1.2 Enumeration of Aerotolerant Heterotrophic Bacteria using Plate Count Agar

This procedure is designed for the isolation and enumeration of heterotrophic bacteria from water and wastewater samples by membrane filtration. The medium used, Plate Count Agar (PCA), is a non-selective nutrient medium designed to culture heterotrophic bacteria. This medium will 1) provide an excellent medium for the isolation of copiotrophic bacteria, 2) allow for correlation of the Gram positive, Gram negative and enteric procedures.

Procedure

Sample preservation and storage. See **Section 9.2.1.**

Preparation. Suspend 8.5 grams of Bacto m-Plate Count Broth and 10 grams of Bacto Agar (Difco Laboratories, Detroit, MI) in 1 L of deionized or distilled water.

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. The final pH of the medium should be 7.0 $\pm$ 0.2 at 25°C.

Sterilization. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Dispensing. Within a LF hood, aseptically dispense 5 mL of the sterile medium into sterile 50 x 9 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Quality Control. The prepared medium should be light amber in color and slightly opalescent. Darkened medium may indicate an increased time or temperature in sterilization and must not be used.

Allow plates to incubate at room temperature, in the dark for 24 hours and inspect for contamination prior to use. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Prepare a positive control using *Staphylococcus epidermidis* (ATCC 12228). The resultant colonies should appear small, round, and smooth with a creamy white color. Record the results in the MPLB. This is not a selective medium, therefore a negative control is not required.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane filters. **See Section 9.2.1.**

Sample application. Follow the Membrane Filtration Method described in Section 9.2.1. Duplicate aliquots of 100, 10, and 1 mL are filtered. Duplicates for sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to plates.

Incubation. Incubate the cultures aerobically at 28 $\pm$ 0.5°C for up to 7 days. Examine the plates after 24 and 48 hours and again after 7 days as some oligotrophic bacteria may require long incubation periods. Count the colonies at the time of each observation.

Interpretation of results. There may be a variety of colony morphologies and pigmentation as this procedure will culture many bacterial types. Count all colonies present. The countable range of colonies is between 20-80 colonies per plate. Plates should be counted using the magnifying lens of a Quebec type colony counter.

Confirmation. Different colonial morphologies will be Gram stained and their cellular morphology will be recorded. A minimum of two plates within the acceptable countable range (20-80 colonies/plate) should be chosen. A minimum of 10% of all colonies for each colonial morphology will be identified.

Note: All colony types will be characterized and/or identified based on the specific sample port location where isolated. For further clarification refer to NASA/MSFC document ED-62 (58-90).

9.2.1.3 Enumeration of Aerotolerant Eutrophic Mesophiles

This procedure is designed for the isolation and enumeration of bacteria associated with the human body. This includes normal flora, opportunistic and pathogenic bacteria. Fastidious organisms with a variety of growth requirements will grow on this medium. The hemoglobin component of the chocolate agar provides iron and the supplement VX provides performed factors which are essential for the culture of some fastidious organisms associated with the normal human flora.

Procedure

Sample preservation and storage. **See section 9.2.1**

Preparation. Suspend 71 grams of Bacto Chocolate Enriched Agar (Difco Laboratories, Detroit, MI) in 1 L of deionized or distilled water.

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. Final pH of the medium should be 7.2 ± 0.2 at 25° C.

Sterilization. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Aseptically add the quantity of medium supplement as directed. **Bacto supplement VX is presterilized and heat-labile.** Return the flask to the magnetic stirrer and mix so that the VX supplement is homogeneously dispersed throughout the medium.

Dispensing. Within a LF hood, aseptically dispense 5 mL of the sterile medium into sterile 50 or 60 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Quality Control. The prepared medium should appear chocolate brown in color.

Allow plates to incubate at room temperature, in the dark for 24 hours, and inspect for contamination prior to use. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Prepare a positive control using Haemophilus haemolyticus (ATCC 33390). The resultant colonies should appear as small, semi-opaque and be gray-white in color. Record the results in the MPLB. This is not a selective medium, therefore a negative control is not required.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane filtration. **See Section 9.2.1.**

Sample application. Follow the membrane filtration method described in **Section 9.2.1**. Duplicate aliquots of 100, 10, and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to plates.

Incubation. Incubate the cultures aerobically at 35 $\pm$ 0.5°C under an increased CO_2 concentration of 5% and relative humidity of 90% for 48 hours.

Interpretation of results. Examine plates and record results after 24 and 48 hours incubation. Magnification may be required to detect small opaque to transparent colonies possibly representing fastidious human pathogens. Count all colonies present on the plate. The countable range of colonies is between 20 and 80 colonies per plate. Plates should be counted using the magnifying lens of a Quebec type colony counter.

Confirmation. Different colonial morphologies will be Gram stained and their cellular morphology will be recorded. A minimum of two plates within the acceptable countable range (20 - 80 colonies/plate) should be chosen. A minimum of 10% of all colonies for each colonial morphology will be identified.

Note: All colony types will be characterized and/or identified based on the specific sample port location where isolated. For further clarification refer to NASA/MSFC document ED-62 (58-90).

9.2.1.4 Enumeration of Gram Negative Bacteria

This procedure is designed for the enumeration of Gram negative bacteria from a water sample using the membrane filtration technique. The medium consists of a tryptose, glucose, and yeast extract Agar base to enrich for the target organisms and crystal violet to selectively inhibit the growth of Gram positive bacteria.

Procedure

Sample preservation and storage. **See Section 9.2.1.**

Preparation. Suspend 8.5 grams of Bacto mPC broth base and 10 grams Bacto Agar (Difco Laboratories, Detroit, MI) in 1 L of deionized or distilled water. Add 1 mL of a stock solution containing 400 mg Bacto crystal violet/100 mL making a final concentration of 4 mg/liter (1:250,000).

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. The final pH of the medium should be 7.0 $\pm$ 0.2 at 25°C.

Sterilization. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Dispensing. Within a LF hood, aseptically dispense 5 mL of the sterile medium into sterile 50 or 60 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Quality Control. The Modified Plate Count Agar with Crystal Violet should appear purple and be clear to slightly opalescent.

Allow plates to incubate at room temperature, in the dark for 24 hours and inspect for contamination prior to usage. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

As a positive control, streak a randomly selected plate using Escherichia coli (ATCC 25922). The resultant colonies should be umbonate in elevation, have a rough surface and edge, and be white to purple in color. As a negative control, streak a second randomly selected plate with Staphylococcus epidermidis (ATCC 12228). There should be little to no growth on this plate. Record the results in the MPLB.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane Filtration. **See Section 9.2.1.**

Sample application. Follow the Membrane Filtration Method described in **Section 9.2.1.** Duplicate aliquots of 100, 10, and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01 and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to plates.

Incubation. Incubate the cultures aerobically at 28 $\pm$ 0.5°C for up to 7 days **(see Method 9.2.1.2).**

Interpretation of results. Examine the plates and record results after 24 and 48 hours incubation and then at 7 days. Pinpoint Gram positive colonies may appear on the plate surface. Compare these colonies to any colonies that may be present on the negative control plate. Disregard those colonies as Gram positive. Count remaining colonies as Gram positive. The countable range of colonies is between 20-80 colonies per plate.

Confirmation. Pick a minimum of 10 suspected Gram negative colonies from the countable plates and perform Gram stains. Gram negative bacteria characteristically stain pink to red.

9.2.1.5 Enumeration of Gram Positive Bacteria

This procedure employs the use of a selective medium for the enumeration of Gram positive bacteria from water samples using the membrane filtration technique. The addition of phenylethanol to a nutritional medium will permit the growth of Gram positive organisms but will inhibit the growth of most Gram negative organisms from a mixed sample.

Procedure

<u>Sample preservation and storage</u>. **See Section 9.2.1.**

<u>Preparation.</u> Suspend 8.5 grams of Bacto mPC broth medium, 10 grams Bacto Agar (Difco Laboratories, Detroit, MI) and 2.5 grams phenylethanol in 1 L of deionized or distilled water.

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. The final pH of the medium should be 7.0 ± 0.2 at 25°C.

<u>Sterilization.</u> Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

<u>Dispensing.</u> Within a Laminar Flow (LF) hood, aseptically dispense 5 mL of the sterile medium into sterile 50 or 60 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

<u>Quality Control.</u> The modified PCA with phenylethanol should appear light amber in color and is slightly opalescent.

Allow plates to incubate at room temperature, in the dark for 24 hours, and inspect for contamination prior to use. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Prepare a positive control using <u>Staphylococcus epidermidis</u> (ATCC 12228). The resultant colonies should appear small, round, and smooth with a creamy white color. Streak a second randomly selected plate with <u>Escherichia coli</u> (ATCC 25922). This plate serves as the negative control. There should be little to no growth on this plate. Record the results for each batch in the MPLB.

<u>Storage.</u> The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane filtration. See **Section 9.2.1**.

Sample application. Follow the Membrane Filtration Method described in **Section 9.2.1**. Duplicate aliquots of 100, 10, and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to plates.

Incubation. Incubate the cultures aerobically at 28 ±0.5°C for 7 days **(see Method 9.2.1.2)**.

Interpretation of results. Examine plates and record the results after 24 and 48 hours and at 7 days. Pinpoint Gram negative colonies may appear on the plate surface. Compare these colonies to any colonies that may be present on the negative control plate. Disregard those colonies as Gram negative. Count remaining colonies as Gram positive. The countable range of colonies is between 20-80 colonies per plate.

Confirmation. Pick a minimum of 10 of the suspected Gram positive colonies from the countable plates and perform Gram stains. Gram positive bacteria characteristically stain purple.

9.2.1.6 Enumeration of Yeasts and Molds

This procedure is designed for the enumeration of yeasts and molds. The medium (Emmons) consists of a low nutrient base with an anti-bacterial agent. In addition, the incorporation of Rose Bengal into the medium will further suppress the growth of bacteria and limit the size and height of mold colonies.

Procedure

Sample preservation and storage. **See section 9.2.1.**

Preparation. Suspend 30 grams Bacto Sabouraud Dextrose Broth and 10 grams agar in 1 L of distilled or deionized water. Add 0.05 g Rose Bengal and heat with continuous stirring allowing the medium to boil to completely dissolve the agar. Promptly remove the medium from the heat when it boils. The final pH of the medium should be adjusted to 7.0 $\pm$ 0.2 at 25° C using 1N NaOH.

Note: Rose bengal is added to this medium as a selective agent for some bacteria and to restrict the size of mold colonies due to the increased incubation time. Some researchers have noted that Rose Bengal may deteriorate to inhibitory products by UV irradiation. Plates should be stored in the dark until just prior to use.

Sterilization. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Antibiotic addition. Prepare the Chloramphenicol by aseptically adding 2 mL ethanol to Bacto Antimicrobic Supplement C. Add entire contents of the rehydrated vial to the cooled medium. **Note: Do not use vials that have been rehydrated for more than 24 hours. Store vials refrigerated in the dark.**

Dispensing. Within a LF hood, aseptically dispense 5 mL of the sterile medium into sterile 50 or 60 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Quality Control. The dehydrated Modified Sabouraud Dextrose Agar should appear light beige. Prepared medium will appear amber to light pink and will be opalescent. Record the result in the Media Preparation Log Book.

Streak a randomly selected plate using *Saccharomyces cerevisiae* (ATCC 9763) as a positive control. The resultant colonies should appear round, smooth, and be pink to red. Prepare a negative control using *Xanthomonas maltophilia* (ATCC 13637). There should be little to no growth on this plate. Record the results for each batch in the MPLB.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane filtration. **See Section 9.2.1.**

Sample application. Follow the Membrane Filtration Method described in **Section 9.2.1**. Duplicate aliquots of 100, 10, and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to plates.

Incubation. Incubate the plates aerobically at 20 ± 0.5°C for 5 days. Examine the plates and record the results after 48 hours, and then daily.

Interpretation of results. Yeast colonies will have a smooth surface and color may vary. Mold colonies will have rough to downy appearance with a variety of colors. Colonies may discolor the medium slightly.

The acceptable countable range of colonies is between 20-60 colonies per plate.

Confirmation. Confirm molds by colonial morphology under binocular dissecting scope (10X). Yeast may be confirmed based on cellular morphology.

9.2.1.7 Enumeration of Legionella

This procedure is designed for the isolation and enumeration of Legionella spp. This method relies on membrane filtration for the initial concentration of Legionella from samples. The filters are subsequently acid treated and aliquots plated directly on a selective enriched agar using the spread plate technique. The BBL BCYE Agar base (Baltimore Biological Laboratories, Cockeysville, MD) consists of a basal medium containing ACES buffer, charcoal, ferric pyrophosphate (0.25 g/L), and alpha-ketoglutarate. A special lyophilized enrichment containing L-cysteine (0.4 g/L) is added. Legionella are fastidious bacteria which are easily overgrown by other bacteria in the environment. As a precaution against this, an acid treatment step is used to reduce the numbers of competing bacteria. A mixture of antibiotics (BBL PAV supplement) which contains Polymyxin B (100,000 units), Vancomycin (5 mg), and Anisomycin (80 mg) is added to each liter of medium to further minimize overgrowth of unwanted bacteria.

Note: Special precaution should be taken when working with any sample suspected of containing Legionella as all species are potential human pathogens. This includes precautions to prevent aerosol formation.

Procedure

Sample preservation and storage. Sodium thiosulfate, at a concentration of 10%, should be added to samples (0.1 mL $Na_2S_2O_3$/100 mL sample) where residual chlorine or other halogen is suspected.

Note: The sodium thiosulfate solution should be added to the sample collection bottles prior to autoclaving. Do not add EDTA to Legionella samples.

Preparation. Suspend 38.3 grams of BBL BCYE agar base (BBL Cockeysville, MD) and 3 grams glycine in 900 mL of deionized or distilled water. The final pH of the medium should be 6.9 ± 0.2 at 25° C. If necessary adjust the pH using 1N KOH. **Do not use NaOH since legionella are sensitive to free sodium ions.** Care should be taken to adjust the pH of the medium to obtain optimal recovery. After adjusting the pH, bring the volume of the medium to 1 L using distilled or deionized water. Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat.

Sterilization. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Enrichment and antibiotic addition. Aseptically rehydrate and add a vial of the BBL cysteine (0.4 g/10 mL) supplement using sterile distilled or deionized water per L of prepared and cooled media. Also rehydrate and add a vial of BBL PAV antibiotic supplement using sterile distilled or deionized water per L of prepared and cooled media.

Dispensing. Within a LF hood, aseptically dispense the sterile medium into sterile 15 x 100 mm Petri dishes. Continually agitate the flask while pouring plates to keep charcoal from settling out of the media. Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Quality Control. The prepared BBL BCYE Agar should appear gray/black in color. Allow plates to incubate at room temperature, in the dark for 24 hours and inspect for contamination prior to usage. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Prepare a positive control using Legionella gormanii (ATCC 33297). Colonies of Legionella spp. should be visible after 2 to 3 days incubation and appear light blue to blue-gray in color and should not discolor medium. Older colonies will become larger, smoother and gray-white in color. Typical cultural response of Legionella should be evident after 48-72 hours. Prepare a negative control using Escherichia coli (ATCC 25922). This control organism should grow but not produce a blue pigment. Record the results obtained from each batch in the MPLB.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Sample preparation. Concentrate maximum amount of water (up to 100 mL), in duplicate, through a 47 mm white, ungridded Nuclepore (Nuclepore Corporation, Pleasanton, CA) membrane having a pore size of 0.2 µM. Multiple membranes may be used, if necessary, and combined to obtain appropriate concentration. Following concentration place filter(s), soiled side down, in a sterile 50 mL centrifuge tube (or similar vessel) with a screw cap containing ten (10) mL of sterile water. Disperse bacteria from filter by vortexing (3 x 30 seconds) or place in a sonic bath for 10 minutes. Repeat the above procedure concentrating duplicate 10 mL aliquots.

Acid treatment. Place 1 mL of each suspension in a sterile 13 x 100 mm screw cap tube containing 1 mL acid treatment reagent (LATR) and vortex for 10 seconds. Let stand for 15 minutes at room temperature and immediately neutralize by adding 1 mL of the alkaline neutralizing reagent (LANR) and vortex for 10 seconds.

Acid treatment reagent:

Solution A: 0.2M KCl (14.9 g/L in distilled or deionized water)
Solution B: 0.2M HCl (16.7 mL/L 10N HCl in distilled/deionized water)

To prepare: Mix 18 parts solution A with 1 part solution B. pH of this solution should be 2.2 at 25°C. Check pH against a pH 2 standard buffer. Dispense in 1 mL volumes into a 13 x 100 mm screw cap tube and sterilize by autoclaving at 121°C for 15 minutes. Label tubes as Legionella Acid Treatment Reagent (LATR).

Alkaline neutralizer reagent:

Stock solution: 0.1N KOH (6.46 g/L in distilled/deionized water)

To prepare: Dilute 10.7 mL of stock solution using distilled or deionized water to 100 mL. Dispense in 1 mL volumes into a 13 x 100 mm screw cap tubes and sterilize by autoclaving at 121° C for 15 minutes. Label tubes as Legionella Alkaline Neutralizing Reagent (LANR).

Note: Equal volumes of LATR and LANR when mixed should result in a pH of 6.9 at 25° C.

Sample application. Inoculate 0.1 mL of the neutralized acid treated suspensions onto the BBL BCYE supplemented agar plates and spread over the entire surface using a glass rod and turntable (See Section 2.3). Sterilize the rod between plating aliquots by soaking in alcohol and then flaming prior to each use. Also prepare serial dilutions, if necessary, from the 1 mL sample aliquots and plate as described above.

Incubation. Incubate the cultures aerobically in an atmosphere containing 2.5% CO2 and a relative humidity of 90%, at 35 $\pm$ 0.5°C for at least 10 days. Examine daily for evidence of growth. Only presumptive colonies of Legionella will be reported.

Interpretation of results. Legionella colonies will appear blue to gray-blue in color and should not discolor this medium. Any colonies fitting this description should be picked for confirmation. The countable range of colonies is between 30-300 colonies per plate. Determine the number of CFUs/100mL using the formula below:

$$\textbf{CFUs/100mL} = \frac{333 \times \text{number of colonies}}{\text{volume of sample filtered (mL)}} \times 100$$

Note: Although this medium is designed for the cultivation and isolation of Legionella spp., other organisms may grow and must be differentiated from the target organism. Also, due to the variety of nutritional requirements of the genus, some strains may be encountered that fail to grow or grow poorly on this medium.

Confirmation. ALL colonies suspected of being *Legionella* spp. should be Gram stained and subcultured to fresh BCYE Agar plates with and without L-cysteine.

Gram negative organisms that grow on BBL-BCYE agar without cysteine may be presumptively identified as Legionella. Definitive identification is performed on all positive presumptive isolates on the basis of growth, morphology, fatty acid profiles, and biochemical and/or immunological reactions.

9.2.1.8 Enumeration of Enteric Bacteria

This procedure is designed for the isolation and enumeration of enteric bacteria from a water sample using the membrane filtration technique. This will include both Gram positive and Gram negative bacteria which can tolerate the selective action of bile salts. The medium consists of a Tryptose, Glucose, Yeast extract Agar base enrichment with Bile Salts No. 3 to inhibit nonenteric bacteria.

Procedure

<u>Sample preservation and storage</u>. **See Section 9.2.1.**

<u>Preparation</u>. Suspend 8.5 grams of dehydrated Bacto m-PC broth medium, 10 grams Bacto Agar and 1.5 grams of Bacto Bile Salts No. 3 (Difco Laboratories, Detroit, MI) in 1 L distilled or deionized water.

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. The final pH of the medium should be 7.0 ±0.2 at 25°C.

<u>Sterilization</u>. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

<u>Dispensing</u>. Within a LF hood, aseptically dispense 5 mL of the sterile medium into sterile 50 or 60 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

<u>Quality Control</u>. The Plate Count Agar with Bile Salts No. 3 should appear slightly amber in color and translucent.

Allow plates to incubate at room temperature, in the dark for 24 hours and inspect for contamination prior to usage. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Streak a randomly selected positive control plate using <u>Escherichia coli</u> (ATCC 25922). The resultant colonies should be umbonate in elevation, have a rough surface and edge, and be off-white in color. As a negative control, streak a second randomly selected plate with <u>Bacillus alvei</u> (ATCC 6344). Little to no growth should be observed. Record the results obtained from each batch in the MPLB.

<u>Storage</u>. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane filtration. **See Section 9.2.1.**

Sample Application. Follow the Membrane Filtration Method described in **Section 9.2.1.** Duplicate aliquots of 100, 10, and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to plates.

Incubation. Incubate the cultures aerobically at 35 ±0.5°C for 48 hours.

Interpretation of results. Examine the plates after 24 and 48 hours. Colonies should appear off-white to yellow and should not discolor the medium significantly. Examine plates closely for the presence of small, off-white, translucent colonies that represent fecal Streptococci. Count all colonies. The countable range of colonies with this medium is between 20-80 colonies per plate.

Confirmation. Confirm enteric bacteria by picking at least 10 isolates and transferring them to Bacto Lauryl Tryptose broth and Enterococci Confirmatory broth. Incubate at 35°C for 24 and 48 hours and examine for growth. Growth in either tube indicates recovery of enteric bacteria.

Note: Lauryl Tryptose broth is prepared by dissolving 35.6 grams Bacto Lauryl Tryptose broth base (Difco Laboratories, Detroit, MI) in 1 L distilled or deionized water. Enterococci confirmatory broth (ECB) is prepared by dissolving 80.4 grams of Bacto ECB (Difco Laboratories, Detroit, MI) into 1 L of distilled or deionized water. Each medium is dispensed into 15 x 125 mm screw cap tubes in 5 mL quantities and autoclaved at 121° C for 15 minutes.

9.2.1.9 Enumeration of Fecal Coliform Bacteria

This procedure is designed for the isolation and enumeration of fecal coliforms from a water sample using the membrane filtration technique. The medium consists of a tryptose, lactose, peptone, yeast extract base enrichment with Bile Salts No. 3 to inhibit Gram positive bacteria. Rosolic acid is added for the color reaction of the fecal coliform bacteria. The elevated incubation temperature enhances selectivity and gives 93% accuracy in differentiating between coliforms from warm-blooded animals and those from other sources.

Procedure

Sample preservation and storage. **See Section 9.2.1.**

Preparation. Suspend 37 grams of dehydrated Bacto mFC Broth Base and 10 grams Bacto Agar (Difco Laboratories, Detroit, MI) in 1 L of sterile distilled or sterile deionized water. Add 10 ml of a 1% solution of Bacto Rosolic acid (Difco Laboratories, Detroit, MI) in 0.2N NaOH. The final pH should be 7.4.

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. **Do not autoclave this medium.**

Dispensing. Within a LF hood, aseptically dispense 5 mL of the medium into sterile 50 or 60 mm diameter Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Quality Control. The mFC agar should be cranberry red in color and slightly opalescent. The medium should appear blue before the addition of the Rosolic acid.

Allow plates to incubate at room temperature, in the dark for 24 hours, and inspect for contamination prior to use. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Streak a randomly selected plate with Excherichia coli (ATCC 25922) to serve as the positive control. The resultant colonies should be umbonate in elevation, have a rough edge, and be blue in color. Streak a second randomly selected plate using Xanthomonas maltophilia (ATCC 13637). This plate will serve as the negative control plate. The resultant colonies should appear round, smooth, and be gray in color. Record the results obtained for each batch in the MPLB.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 14 days. Plates should be stored in the dark and allowed to equilibrate to room temperature just prior to use.

Membrane filtration. **See Section 9.2.1.**

Sample Application. Follow the Membrane Filtration Method described in **Section 9.2.1**. Duplicate aliquots of 100, 10, and 1 mL are filtered for each sample. Duplicates for sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to plates.

Incubation. Place the prepared cultures in water tight plastic bags. Submerge the bags containing plates in a water bath at 44.5 $\pm$ 0.2^{o}C for 24 $\pm$ 2 hours. The bags should be anchored under the water to maintain the critical temperature requirements. **Place all prepared plates containing filters in the water bath within 30 minutes after filtration.**

Interpretation of results. Examine the plates after 24 hours. Fecal coliform colonies should appear blue in color and nonfecal colonies should appear gray. Few nonfecal colonies should be present on the plates due to the inhibitory effect of the medium and incubation temperature. The countable range of colonies with this medium is between 20-60 colonies per plate.

Confirmation. Verify fecal coliforms by picking at least 10 isolates exhibiting a blue color and transfer to EC broth. Incubate at 44.5 $\pm$ 0.2^{o}C for 24 hours. Confirmation of fecal coliforms is indicated by gas accumulation in the Durham tube.

Note: EC broth is prepared by dissolving 37 grams Bacto EC broth base (Difco Laboratories, Detroit, MI) in 1 L of distilled or deionized water. The medium is brought to a boil with continuous stirring, dispensed into 15 x 125 mm screw cap tubes in five mL quantities, and autoclaved at 121^{o} C for 15 minutes.

9.2.2 Direct Enumeration Using the Spread Plate Technique

Procedure

1) Label petri dishes with the sample ID, date, dilution, analyst's initials and any other pertinent information as specified in the laboratory's SOP document. Media type and batch number should have previously been recorded on the plates.

Note: The plated media must be predried. This may be accomplished by incubating the plates with lids on at 55°C for 12-18 hours. A 2-3 gram weight loss is not uncommon.

2) Vortex the sample to be plated for 10 seconds to evenly distribute the bacteria.

3) Pipette 0.1 mL of the sample directly onto the agar surface.

4) Using a bent sterile glass rod, distribute the inoculum over the surface of the medium by rotating the dish on a turntable. Allow the inoculum to be completely absorbed into the media before transferring the plates to the incubator. The spreading rods are sterilized between sample applications by soaking in 90% (v/v) ethanol and flaming prior to use.

5) Incubate at the specified temperature and time as dictated by the procedure.

6) Count colonies as prescribed by the specific procedure (see **Section 9.2.1.7**). The countable range using the spread plate technique is between 30-300 colonies per plate (15 x 100 mm).

7) Calculate the number of CFUs/100 mL as described by the procedure and dilutions being used (see Section 2.1.7).

Quality Control:

Prepare at least two replicate plates for each sample aliquot and dilution being used.

9.3 Culture of Anaerobic Bacteria

9.3.1 Enumeration Of Anaerobic Bacteria

This procedure is designed for the cultivation and enumeration of non-stringent heterotrophic anaerobic bacteria from a water sample using the membrane filtration technique. The medium consists of a nutritious base and thioglycolate which helps insure a reduced anaerobic medium.

Procedure

Sample preservation and storage. Sodium thiosulfate at a concentration of 10% should be added to samples (0.1 mL $Na_2S_2O_3$/100 mL sample) where residual chlorine or other halogen is suspected.

Samples suspected of containing copper, zinc, or heavy metals should be additionally preserved using ethylenediaminetetraacetic acid (EDTA) as a chelating agent. 0.3 mL of a 15% solution of EDTA, adjusted to pH 6.5, is used per 100 mL of sample.

Note: The sodium thiosulfate and EDTA solutions should be added to the sample collection bottles prior to autoclaving. These solutions may be combined and added as a single solution.

Ideally, samples should be processed on-site immediately following collection. A small hand-held type vacuum pump within a nitrogen filled glove bag may be used for filtration purposes. Samples should be processed within four (4) hours following collection.

If samples are to be transported to the laboratory prior to processing, a transport medium should not be used. In addition, samples suspected of containing anaerobic bacteria should be transported at ambient temperature.

Preparation. Suspend 58 grams of Bacto Brewer Anaerobic Agar in 1 L of oxygen free deionized or distilled water. (Difco Laboratories, Detroit, MI.)

Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. The final pH of the medium should be 7.2 ± 0.2 at 25^{o} C.

Note: Oxygen free water may be prepared by boiling the water for five minutes prior to use. Care should be taken that a sufficient quantity of water is allowed to boil to account for loss due to evaporation during boiling.

Sterilization. Autoclave at 121°C for 15 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 15 minutes between preparation and autoclaving. Do not allow the medium to stand for more than 1 hour at 50°C before pouring plates.

Dispensing. Within a LF hood, aseptically dispense 5 mL of the sterile medium into sterile 50 or 60 mm diameter x 9 mm Petri dishes.

Allow the agar to solidify and cool in the LF hood with the lids slightly ajar to prevent the excess accumulation of moisture in the plates.

Plates poured should be stored **immediately** upon solidification for 1-2 days in the reduced atmosphere of the anaerobe chamber or anaerobe jar prior to use.

Quality Control. The prepared medium should initially appear beige becoming red in color due to aeration upon standing. **Do not use any plates which have turned red.**

Allow plates to incubate at room temperature, in the dark for 24 hours under anaerobic conditions and inspect for contamination prior to use. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Streak a randomly selected positive control plate using Clostridium beijevinickii (ATCC 17795) and incubate under anaerobic conditions. The resultant colonies should be circular to irregular, entire to scalloped, flat to raised, translucent, gray in color, shiny and smooth. As a negative control, streak a second randomly selected plate with Xanthomonas maltophilia (ATCC 13637) and incubate under anaerobic conditions. There should be little to no growth on this plate. Record the results obtained for each batch in the MPLB.

Storage. The prepared plates may be stored at 4°C **under anaerobic conditions** for up to 10 days. Plates should be stored in the dark and allowed to equilibrate to room temperature **under anaerobic conditions** just prior to use.

Sample application. All work is to be conducted within a reduced oxygen atmosphere. Follow the Membrane Filtration Method described in **Section 9.2.1**. Duplicate samples of 100 (or maximum filterable volume), 10, and 1 mL are filtered. Duplicate sample dilutions of 0.1, 0.01, and 0.001 mL are also prepared and filtered. Aseptically transfer the filters to the plates under anaerobic conditions.

Note: If samples are transported to a laboratory prior to filtration, a transport medium should not be used.

Dilution and rinse water. The dilution and rinse water for filtration purposes will be held for 2 days under an anaerobic atmosphere. If milk dilution bottles are used for diluent water storage then the caps should be loosened so that the contents will outgas any oxygen present.

Incubation. Incubate the cultures anaerobically at 35 ± 0.5°C for 7 days. The anaerobic environment can be produced by the use of a commercially available anaerobe system consisting of an anaerobe jar, anaerobe gas packs, and palladium catalyst. An Anaerobic chamber (glove box) may also be used. The use of an indicator is required to insure achievement and maintenance of a reduced environment.

Interpretation of results. This method will allow the recovery of both anaerobic and facultatively anaerobic bacteria. Examine the plates after 48 hours, 5 days, and 7 days. The countable range of colonies with this medium is between 20-60 colonies per plate.

Confirmation. All isolates must be confirmed as anaerobes. Colonies are picked and streaked on duplicate Brewers Anaerobic Agar plates. One plate is incubated aerobically and the other plate incubated under anaerobic conditions. Anaerobes will not grow on the plate incubated aerobically. All isolates which grow **only** under anaerobic conditions will be identified.

9.3.2 Presence of Anaerobic Bacteria

The following is a procedure to perform enrichment and isolation of nonstringent anaerobic mesophilic bacteria. Nonstringent anaerobic bacteria are those that may survive some exposure to, but will not grow in, an oxygenated environment. The fluid thioglycollate medium used in this procedure supports the growth of a wide variety of fastidious microorganisms having a range of growth requirements. The incubation time and temperature are optimized for Clostridia.

Procedure

Enrichment medium preparation. Suspend 59.6 grams of Bacto Fluid Thioglycollate medium (Difco Laboratories) in 1 L deionized or distilled water. This is equivalent to a 2x solution.

Heat with continuous stirring allowing the medium to boil to dissolve completely. The final pH of the medium should be 7.1 $\pm$ 0.2 at 25^{o}C.

Dispensing. Dispense 100 mL of the medium into a clear 250 mL serum bottle (Wheaton, model # 223950).

Sterilization. Sterilize in the autoclave for 15 minutes at 15 lbs pressure (121^{o}C).

Purging medium. After autoclaving (while media is still hot) aseptically install and crimp septum and insert the gas purge apparatus (see the figure below). The apparatus consists of a purge gas filter (modified syringe) needle and an outgassing (venting) filter and needle. The purge gas filter and needle must be sterilized by autoclaving before insertion into medium. A manifold can be constructed so that multiple bottles can be simultaneously purged. Purge with purified nitrogen using the point of use filter until the medium cools.

Note: Only a small N_2 pressure/flow needs to be applied (a few PSI).

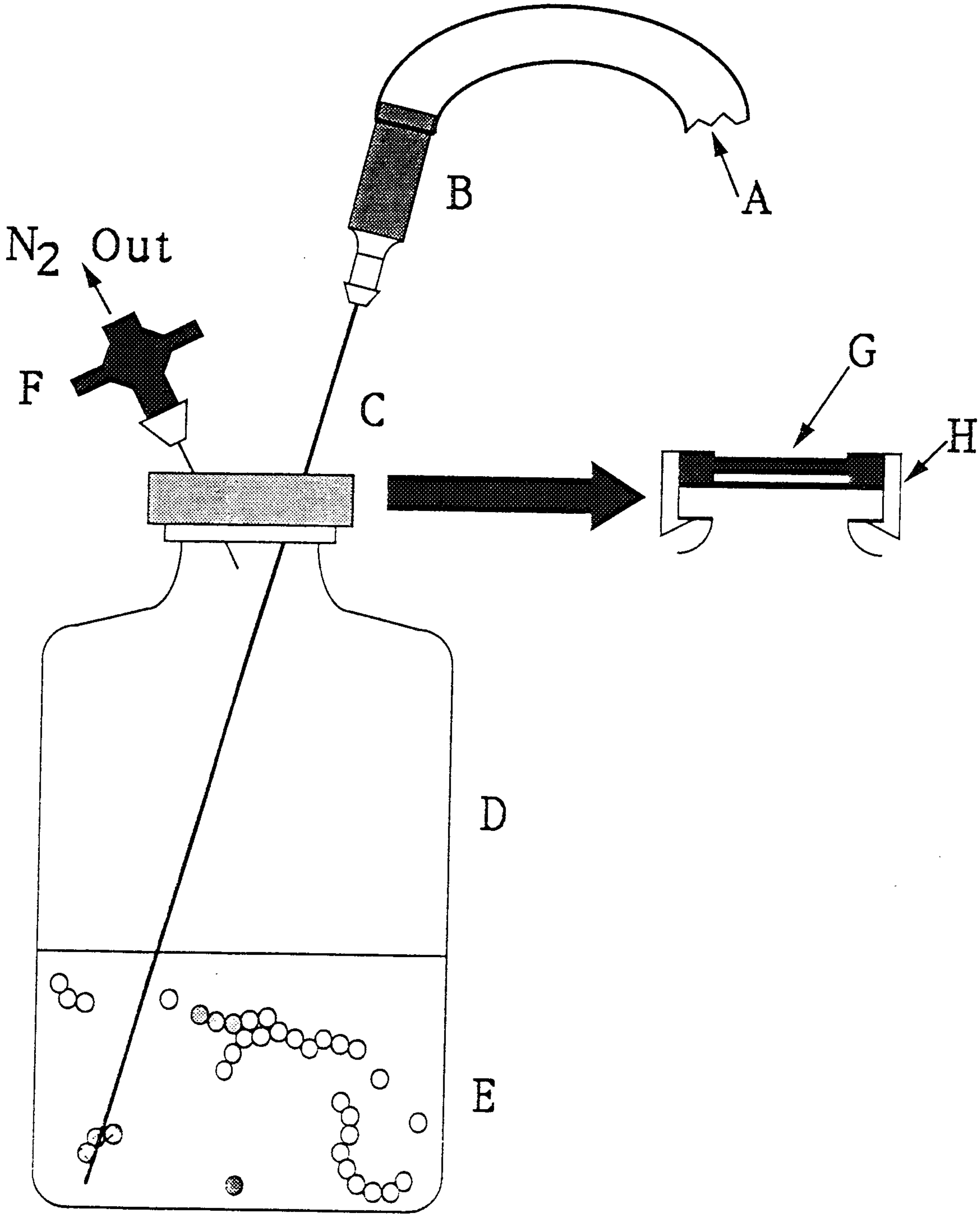

Figure 9-1 GAS PURGING APPARATUS

LEGEND FOR ENRICHMENT MEDIA PREPARATION FIGURE

A= PURGE GAS INLET TUBING, butyl rubber, 1/4 inch ID, Fisher Cat. no. 14-168B

B= PURGE GAS POINT OF USE FILTER, 2-cc Luer-Lok syringe, barrel packed with cotton, flange removed so that 1/4 inch ID tubing can be fitted. Fisher Cat. no. 14-823-10A

C= PURGE GAS INLET NEEDLE, 22-gauge deflected non-coring septum penetration point needles w/ Luer-Lok connection. Popper & Son Cat. no. 7176

D= SERUM BOTTLE, 250 mL. Wheaton Cat. no. 223950

E= ENRICHMENT MEDIUM

F= PURGE GAS OUTLET NEEDLE and FILTER, 26 gauge, 5/8 inch needle B-D Precision Glide, Fisher Cat. no. 14-826-6A fitted to a 0.45 um porosity, filter assembly (Acro LC3S, Gelman Sciences, product no. 4440). These filters must be sterilized by autoclaving before use.

G= SEPTUM, silicone/TeflonTM, 30 mm diameter. Wheaton Cat. no. 224174

H= CRIMP CAP, aluminum septa seals for serum bottles. Wheaton Cat. no. 224127

Enrichment medium quality control. The prepared medium should be light straw-colored and will be slightly opalescent, with the upper 10%, or less, of the medium pink.

Allow bottles to incubate in the dark, at room temperature, for 48 hours under anaerobic conditions (anaerobe chamber or anaerobe gas pack containers) and inspect for contamination. Record the number of contaminated bottles in the MPLB. If more than 10% of the bottles are contaminated, discard the entire batch.

Inoculate a randomly selected bottle of prepared medium from each batch using Bacteroides melaninogenicus (ATCC 25845) and incubate under anaerobic conditions. This bottle will serve as the positive control. Good to excellent growth should be visible after 18-48 hours. Inoculate a second randomly selected bottle from each batch using a negative control organism Micrococcus luteus (ATCC 533). Record the results obtained for each batch in the MPLB.

Caution: As headspace is limited in these enrichment bottles and many anaerobes produce copious quantities of gas, a venting needle fitted with sterile filter is advised. Use a 26 gauge, 5/8 inch needle (B-D Precision Glide, Fisher Cat. no. 14-826-6A) for media quality control check venting and inoculations. This should minimize coring and leaking septa.

Storage. The prepared enrichment medium should be stored in the dark at 15-30°C.

Note: If upon storage, more than 10% of the uppermost portion of the medium has changed to a pink color, it is not suitable for use and should be discarded.

Sample Collection. As the 18 gauge sample port needle will most probably core the enrichment bottle septum, cautions must be taken to "protect" the reduced nature of the sample + enrichment medium.

1) Wipe the bottle septum with a solution of 3% H_2O_2 using a sterile cotton ball. Insert venting sterile needle with filter into the edge of the septum using 26 gauge, 5/8 inch needle (B-D Precision Glide, Fisher Cat. no. 14-826-6A) fitted to a 0.45 µm porosity, filter assembly (Acro LC3S, Gelman Sciences, product no. 4440). **These filters must be sterilized by autoclaving before use.**

2) Collect the sample. The bottle will be filled with sample water up to the 200 mL mark (100 mL of 2X medium and 100 mL of sample).

3) As the sample port needle (18 gauge) will most probably core the enrichment bottle septum, immediately insert into the septum hole a sterile 18 gauge, 1.5 inch length needle (B-D Precision Glide, Fisher Cat. no. 14-826-5d)

4) Remove 26 gauge needle/vent installed in (1).

5) Aseptically exchange the 0.45 um porosity, filter assembly (Acro LC3S, Gelman Sciences, product no. 4440) from the 26 gauge needle/vent to the 18 gauge vent needle installed in (3). This should be done quickly to minimize contamination.

6) Immediately place the enrichment bottle with vent into anaerobe glove bag or, Gas Pack Pouch System (BBL Cat. no. 60651) sealed with the Gas Pack Pouch Sealing Bar (BBL Cat. no. 60652).

7) Place in darkness immediately.

Caution: Never allow media to contact filter vent as this may impair its function. Bottles should always be handled, stored, or shipped in an upright manner.

Enrichment incubation. Incubate the inoculated bottles anaerobically at 35 ±0.5°C for 4 days.

Interpretation of results. Examine the bottles after 48 hours and 4 days for turbidity (growth). Report results as positive or negative. If positive, proceed with isolation.

Isolation medium preparation. Prepare Brewer Anaerobic Agar plates (Difco) as directed in PROCEDURE 3.1.

Isolation medium sterilization. **See Procedure 9.3.1.**

Isolation medium dispensing. **See Procedure 9.3.1.**

Isolation medium quality control. **See Procedure 9.3.1.**

Diluent. Peptone water diluent is to be used for this procedure. Prepare as directed in **Procedure 9.2.1.**

The diluent will be held for 2 days under an anaerobic atmosphere. If milk dilution bottles are used for diluent storage, then the caps should be loosened so that the contents will outgas any oxygen present.

Isolate development. Isolates are to be developed by the spread plate technique utilizing Brewer Anaerobic Agar plates. This procedure is to be conducted within an anaerobic glove bag. Spread 0.1 mL volumes onto duplicate plates representing sample volumes of 10^2 - 10^9 of the enrichment culture.

Isolation plate incubation. Plates are to be incubated at 35°C for 7 days.

Confirmation. Isolated colonies are picked and streaked onto two Brewer Anaerobic Agar plates. One plate is incubated aerobically and the second plate incubated under anaerobic conditions. Anaerobes will not grow on the plate incubated aerobically. All isolates, which grow **only** under anaerobic conditions, will be identified.

9.4 Direct Total Microbial Count

9.4.1 Direct Epifluorescent Filter Technique (DEFT)

The following procedure describes the direct microscopic count of bacteria utilizing a fluorochrome and the membrane filtration technique. This technique is designed to determine the total number of bacteria in water samples.

Procedure

1) Select a slide with the appropriate fluorescent stain. Check to see that the proper filter pack is in place in the scope.

2) Low fluorescence immersion oil and the 100x objective are used for counting.

3) Randomly select a field and count all the bacteria seen in that field, starting at the top of the field, counting left to right. Be sure to count only those objects with "bacterial shapes". Do not count fluorescent debris. Record results.

4) Change randomly to another field and count again in the same manner.

5) Determine the average number of bacteria per field.

6) Determine the total number of fields to count using the following table:

Average number of bacteria per field	Minimum number fields to count
≥ 15	10
10-14	25
6 -9	50
3-5	75
≤ 2	100

7) Calculate the total number of bacteria in the water sample using the following equations:

Total Count = Mean bacteria count /field x Microscopic Factor (MF)

where,

$$\mathbf{MF} = \frac{\text{Area of membrane through which sample is filtered (mm}^2\text{)}}{\text{Microscopic field [or grid] area (mm}^2\text{) x Sample volume (mL)}}$$

9.4.1.1 Acridine Orange Staining For Epifluorescence Microscopy

This procedure utilizes the fluorochrome acridine orange to stain nucleic acids within microbial cells. When coupled with membrane filtration and epifluorescence microscopy, a rapid total direct count of microbial cells in aqueous solutions is feasible.

Sample fixation. Prepare the glutaraldehyde stock fixative solution fresh daily. The fixative stock solution is 5.0% (W/V) glutaraldehyde in phosphate buffer (see composition below). At the time of collection, add the glutaraldehyde fixative solution to the sample equivalent to 10.0% (V/V). The final concentration of glutaraldehyde is 0.5%.

Sample storage. Preserved (fixed) samples may be stored in the refrigerator for up to 21 days.

Procedure

1) Prepare the phosphate buffer for use in this procedure by dissolving 13.6 g KH_2PO_4 in distilled or deionized water and dilute to 500 mL. Adjust to pH 7.2 if necessary. Bring volume to 1 L using distilled or deionized water. Autoclave and store prepared buffer at $4^{o}C$ between uses. Filter daily aliquots through a 0.2 μm filter before use.

2) Prepare fluorochrome as 0.1% (w/v) acridine orange in phosphate buffer. Filter this solution at the time of use through 0.2 μm disposable sterile syringe filter. Store in a light-proof container at $4^{o}C$

3) Use a 25 mm cellulose backing-filter (Millipore Corporation, Bedford, MA) having a 0.45 μm porosity. Dampen this filter first with filtered rinse water so that it adheres to the filtration base. Place a Nuclepore (Nuclepore Corporation, Pleasanton, CA) pre-stained (black) polycarbonate filter, 25 mm, diameter having a 0.2 μm porosity on top of the backing/filter. Assemble the filtration apparatus.

4) Determine the volume of sample to be filtered that is required for direct counting. Twenty mL of clean or potable water is usually sufficient. Volumes up to 25 mL may be added directly to the filter within the apparatus. If dilutions need to be made use phosphate buffer. For larger volume requirements 25 mL aliquots may be consecutively added and filtered through the same filter.

5) Add Acridine Orange stock solution to the sample (or dilution) at a ratio of 1:1 (v/v).

6) Stain for 2 minutes.

7) Add phosphate buffer to the stain/sample equivalent to a final ratio of 3:1:1 (3 parts buffer to 1 part stain to 1 part sample).

8) Filter with vacuum (approximately 13 kPa).

9) Rinse with a volume of phosphate buffer equivalent to one half the total volume of the: stain solution + sample + buffer.

10) Filter with vacuum (approximately 13 kPa).

11) Remove the Nuclepore filter from base and backing-filter by its edge and air dry.

12) Place a small drop of low fluorescence immersion oil on a clean slide. Place the filter on the slide **so that the sample side faces the objective lens.** Add a small drop of oil to the filter and overlay with coverslip.

13) Examine filter surface with an epifluorescence microscope and oil immersion objective utilizing low fluorescence oil.

14) Determine average number of cells per field and calculate the number of cells/100 mL as specified in Procedure 2.2.

9.5 APPLICABLE DOCUMENTS

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Appendix 1

Site Inspection Standard Operating Procedure

Site Inspection Standard Operating Procedure

Site inspections will be carried out by the ACC during testing on a random basis, but will be conducted for a minimum of 50% of the test days for a particular test. Site inspections are informal, and are conducted in such a way as to avoid hindering test personnel or the test itself. A formal site audit may be performed if the ACC determines gross negligence on the part of any test team, or member. This negligence will be determined based on the results of the informal site inspections.

During a site inspection, the ACC will discuss progress of the test with test personnel. In addition, the ACC will view the work of the Samplers, the Data Custodian Personnel, and any other personnel involved with the sampling and distribution of test samples. Based on the results of these general investigations, the need for more specific inspections may be determined.

The ACC will, during all site inspections, verify compliance with the AC Plan. Any deviation from authorized procedure will be noted by the ACC, and discussed with the personnel involved. For serious deviations, the incident will be reported to ED/62 in written form. In addition, the ACC will keep ED/62 informed of efforts to resolve such problems.

Appendix 2

Analytical Control Sample Preparation
Standard Operating Procedure

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SAMPLE PREPARATION PROCEDURE

NASA/MSFC WRT QUALITY CONTROL SAMPLES

Supplement

to the

Analytical Control Test Plan

for the

Water Recovery Systems Test

Prepared for:

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June 6, 1990

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June 22, 1990

SAMPLE PREPARATION PROCEDURE

NASA/MSFC WRT QUALITY CONTROL SAMPLES

1.0 GENERAL

The purpose of this procedural document is to define specifications for quality control (QC) sample preparation for water samples used in the analysis of certain chemical and physical parameters. Stock certified reference materials have been purchased from Environmental Resource Associates (ERA) in Arvada, Colorado. Each standard has been assigned a lot number by ERA. ERA has also provided certified values and advisory ranges for each lot and instructions on how to prepare the standards for analysis. The procedures for preparation used in this document are taken directly from the ERA instructions. A series of QC samples for chemical and physical parameters will be prepared to correspond to each batch of water samples collected in the NASA Water Recovery Testing (WRT) effort. When testing is in progress a daily sample schedule will be developed by the Analytical Control Coordinator (ACC). This schedule will specify the number and types of QC samples required. The QC samples will be prepared by a trained analyst capable of making dilutions and volumetric measurements.

2.0 GLASSWARE

All glassware used in QC sample preparation will be clean and free of contamination to the maximum extent possible. All pipettes, graduated cylinders, and volumetric flasks will be rated Class A. Volumetric flasks have been purchased specifically for this program and will be used on this program only. Furthermore, volumetric flasks will be segregated for each standard category and be reused only in that particular category. No volumetric flasks will be used other than those dedicated specifically to the WRT program and the correct standard category. Scratched or broken glassware will be discarded.

2.1 Glass Specifications

Borosilicate glassware will be used for all laboratory operations, except where individual methods specifically indicate use of alternate materials. These include Kimax and Pyrex brands or equivalent. It should be remembered that borosilicate glassware is not completely inert. For this reason, standard solutions of boron, silica, and the alkali metals will be stored in polyethylene containers. Laboratory glassware will serve three basic functions: storage of reagents and samples, measurement of solution volumes, and confinement of reactions.

Dilute metal solutions are prone to plate out on container walls over time while being stored. Therefore, dilute standard metals solutions will be freshly prepared prior to analysis.

All volumetric glassware purchased for this program will meet Federal specifications for designation as Class A glassware. Class A glassware does not require recalibration by laboratory personnel prior to use. Should it become necessary to recalibrate glassware, directions are included in the EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories (EPA-600/4-79-019), March, 1979.

Laboratory personnel will be thoroughly familiar with and consistently demonstrate approved techniques for usage of volumetric glassware. Proper techniques are illustrated in the EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories (EPA-600/4-79-0119), March, 1979.

2.2 Glassware Cleaning

Methods for cleaning glassware will take into consideration both the substances which are to be removed and the laboratory analyses to be performed. Special cleaning requirements for specialized specific determinations. Special requirements for specialized glassware, fritted ware, and filters are included in the EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories, (EPA-600/4-79-019), March, 1979.

Removal of Water Soluble Substances

Glassware and porcelain vessels will be cleaned with a nondepositing soap, i.e. Alconox, and rinsed a minimum of three times in tap water, followed by two rinses in deionized water.

Removal of Water Insoluble Substances

Laboratory glassware may require the use of detergents, organic solvents, dichromate cleaning solution (11 ml concentrated H2SO4 added slowly with stirring to 35 ml of saturated sodium dichromate solution), nitric acid, or aqua regia (25% by volume concentrated HNO3 in concentrated HCL). The use of NoChroMix is gaining wide acceptance as a substance for dichromate cleaning solution due to the growing concerns of potentially carcinogenic residues which can be produced by the latter. Note: NoChroMix should not be stored in a sealed container. Greasy spots may also be removed by using acetone, alcoholic potassium hydroxide, or a warm solution of NaOH (1g per 50 ml water, left to stand 10-15 minutes, followed by a water rinse, a dilute HCL rinse, and final deionized water rinses).

This laboratory will generally use the following method:

(1) Soak in dichromate cleaning solution or substitute.
(2) Wash with a non-depositing detergent.
(3) Rinse with tap water (3x).
(4) Rinse with ultrapure water (2x).

Cleaning for Trace Metals Determinations

For trace metal determinations one primary concern is contamination. Dust in the laboratory environment, impurities from laboratory equipment, and residues from primary cleaning procedures are sources of potential contamination. The sample bottle, whether borosilicate glass, polyethylene, polypropylene, or Teflon, should be thoroughly washed with detergent and tap water, rinsed with 1:1 Nitric Acid (trace), deionized water, 1:1 Hydrochloric Acid (trace), and finally three rinses with deionized distilled water, in that order. After washing, containers should be used immediately or for short term storage covered with lids or parafilm to prevent atmospheric contamination.

Cleaning of Glassware for Organic Determinations

Glassware used for trace organic constituents should be washed at least 15 minutes in chromic acid, NoChroMix, or other appropriate acid (nitric, sulfuric, etc) to destroy organic residues. They should then be rinsed thoroughly in tap water and finally distilled deionized water. Glassware may be dried with redistilled nanograde acetone when needed for immediate use; otherwise it is oven or drip dried. This glassware should be stored inverted or covered with aluminum foil to prevent dust contamination.

Specialized Cleaning

Special cleaning requirements exists for particular types of vessels and for glassware to be used for specific determinations. Special requirements for specialized glassware, fritted ware, and filters are included in the EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories, (EPA-600/4-79-019), March, 1979. Some of the most frequently occurring special cleaning requirements are listed in Table 1.

TABLE 1 SPECIAL CLEANING REQUIREMENTS

	Washing	Rinsing	Drying/ Storage	Precautions
Absorption Cells	Detergents/ organic solvents. Do not use dichromate solution.	HNO3 rinse, alcohol, or acetone.	Remove any film. Store protected from dust.	Soaking may produce etching. Cells must be checked for equivalence after washing. Compare transmittrance or OD values.
Trace metals/ Lead analysis		1:1 Nitric acid, followed by 10-12 tap water rinses, followed by 4 ultrapure water rinses.		
Phosphate determination		Rinse thoroughly with ultrapure water.		Do not use phosphate-containing detergent.
Ammonia/Kjeldahl Nitrogen Analysis		Rinse with ammonia free water.		
Trace organic substituents/ chlorinated pesticides	15 minute wash with dichromate solution.	Rinse with acetone.	Dry at $375^{o}C$ for 4 hours.	Store inverted and protected from dust.
Collection bottles and liners for reuse (not recommended)	Wash with dichromate solution.	Rinse with ultrapure water, then several rinses with redistilled solvent such as acetone, hexane, ether, or chloroform.	Store inverted and protected from dust. Store liners & caps in sealed containers.	
Caps for collection of organics	Detergent wash.	Dist. HOH & redis. solv.	Sealed containers.	

2.3 Sample Bottles

Special sample bottles have been purchased for the QC samples to be prepared for the WRT program. Bottles sizes of 40 mL, 125 mL, 250 mL, 500 mL, and 1000 mL were obtained from I-Chem Research and meet cleanliness standards published by the U.S. Environmental Protection Agency (EPA). Bottles are packaged in cases and are accompanied by chain-of-custody documentation originated by the vendor. Chain-of-custody is maintained in the laboratory by (i) storing opened cases in restricted access areas (ii) inventorying and documenting partial cases prior to storage and subsequent use and (iii) sealing opened cases with tamper-indicating seals. Bottles from cases showing evidence of unauthorized use are not used.

3.0 WATER PURITY

The quality of ultrapure water used for this program is assured by a rigorous maintenance schedule for deionization and reverse osmosis equipment. Confirmation of ultrapure water quality is accomplished by periodic analysis of the water for pH, conductivity and total organic carbon (TOC). Documentation regarding water system maintenance and analytical results are recorded in a log book.

4.0 SAMPLE DOCUMENTATION

Prepared samples are identified using numbers assigned by the data custodian, a representative of MSFC Test Laboratory (EL/64). The ACC is responsible for ensuring that the proper sample numbers are affixed to the proper sample bottle.

The ACC maintains sample number integrity by entering assigned sample numbers and other relevant data such as ERA lot number, I-Chem lot number and required dilution volume into a logbook.

Once samples have been prepared, the laboratory technician originates the chain-of-custody documentation which will accompany the samples in transit to the data custodian. **Figure 1 of this appendix** illustrates the chain-of-custody form which is used for all samples prepared for this program.

4.1 QC Sample Logbook

The ACC will maintain a logbook for all QC samples used in the WRT program. The information recorded in the logbook includes: Sample number, ERA standard lot number bottle code, ERA standard category, sample volume, sample bottle volume and case code number, the initials of the analyst who prepares the sample, the date prepared, and the date delivered to NASA/MSFC.

4.2 Chain of Custody Form

An internal chain-of-custody form **(Appendix 2, Figure 1)** will be maintained for each daily allotment of QC samples. The preparer will initiate the form as samples are prepared. The ACC will deliver the samples to the Data Custodian at NASA, who will sign the form upon assuming custody. The ACC will return the form to the internal filing system. The information on the form includes: date, sample numbers, preparers signature, ACC signature, and Data Custodian signature.

4.3 QC Sample Labels

Sample labels will be provided to the ACC by the Data Custodian 24 hrs before test day start up. The preparer will affix the appropriate label to each bottle prior to preparation. The ACC or analyst will enter the following information on the sample labels prior to affixing them to the sample bottles: sampler's initials, sample date/time, field tracking number (to be provided by Test Samplers), and preservative (where required).

5.0 STORAGE OF CONCENTRATED AND PREPARED STANDARDS

All concentrated and prepared standards are organized by lot number and parameter and stored in a locked refrigerator at 4°C. The ACC maintains possession of refrigerator keys and is responsible for verifying that the refrigerators are locked when leaving the laboratory facility.

Prepared standards (samples) are returned to the refrigerator immediately following preparation. Once all necessary samples have been prepared, the samples are removed from the refrigerator and packed into plastic coolers for shipment to NASA/MSFC. Only "blue ice" is used in coolers to maintain shipping temperatures of 4°C. Because many samples have limited holding times, all samples are delivered to the NASA/MSFC data custodian within 12 hours of preparation.

6.0 PREPARATION OF QC SAMPLES

Quality control samples are prepared by diluting ERA stock solutions with ultrapure water in accordance with ERA instructions. Sample aliquots are transferred to labeled sample bottles immediately following dilution and mixing. Chemical preservatives are added if samples collected during water recovery system testing receive a chemical preservative.

For selected parameters, dilution of ERA materials is not required. In these instances, measured volumes of ERA solutions are transferred directly to labeled sample bottles.

6.1 Minerals and Hardness

The minerals and hardness standards furnished by ERA do not require dilution prior to use. The laboratory technician prepares Quality Control samples for minerals and hardness by measuring 100 mL of solution into a graduated cylinder and pouring the cylinder contents into a labeled sample bottle. The capped sample bottles are placed in the refrigerator at 4°C pending shipment to the NASA/MSFC Data Custodian. Any unused solution is discarded.

6.2 Demand

Preparation of Demand Quality Control Samples is accomplished by volumetric pipeting 5.0 mL of ERA Demand Concentrate into a 500 mL volumetric flask. Ultrapure water is added to the mark. The flask is inverted several times to facilitate mixing. Following thorough mixing, Demand samples are withdrawn from the volumetric flask and transferred to labeled sample bottles. Samples bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.3 Nutrients

Preparation of Nutrient Quality Control Samples is accomplished by volumetric pipeting 5.0 mL of ERA Nutrient Concentrate into a 500 mL volumetric flask. Ultrapure water is added to the mark. The flask is inverted several times to facilitate mixing. Following thorough mixing, Nutrient samples are withdrawn from the volumetric flask and transferred to labeled sample bottles. Sample bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.4 Cyanide

Preparation of Cyanide Quality Control Samples is accomplished by volumetrically pipeting 5.0 mL of ERA Cyanide Concentrate into a 500 mL volumetric flask. Ultrapure water is added to the mark. The flask is inverted several times to facilitate mixing. Following thorough mixing, Cyanide samples are withdrawn from the volumetric flask and transferred to labeled sample bottles. Sample bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.5 Phenol

Preparation of Phenol Quality Control Samples is accomplished by volumetrically pipeting 5.0 mL of ERA Phenol Concentrate into a 500 mL volumetric flask. Ultrapure water is added to the mark. The flask is inverted several times to facilitate mixing. Following thorough mixing, Phenol samples are withdrawn from the volumetric flask and transferred to labeled sample bottles. Sample bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.6 Trace Metals

Preparation of Trace Metals Quality Control Samples is accomplished by volumetrically pipeting 5.0 mL of ERA Trace Metals Concentrate into a 500 mL volumetric flask containing 250 mL ultrapure water and 25 mL concentrated HCL. Once acidification is complete, the flask contents are mixed, additional ultrapure water is added to the mark. The flask is inverted several times to facilitate mixing. Trace Metal samples are withdrawn from the volumetric flask and transferred to labeled sample bottles. Sample bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.7 Residual Chlorine

Preparation of Residual Chlorine Quality Control Samples is accomplished by volumetrically pipeting 1.0 mL of Concentrate into a 1 liter volumetric flask. Ultrapure water is added to the mark. No preservative is required. The flask is inverted several times to facilitate mixing. Following thorough mixing, samples are withdrawn from the volumetric flask and transferred to labeled sample bottles. Sample bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.8 Turbidity

Preparation of Turbidity Quality Control Samples is accomplished by volumetrically pipeting 5.0 mL of Turbidity Concentrate into a 500 mL volumetric flask. The turbidity concentrate is well shaken before an aliquot is withdrawn. Ultrapure water is added to the mark. No preservative is required. The flask is inverted several times to facilitate mixing. Following thorough mixing, samples are withdrawn from the volumetric flask and transferred to labeled sample bottles. Sample bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.9 Volatiles

The concentrate is equilibrated to ambient temperature. Five µL of the concentrate are transferred into a 100 mL volumetric flask containing approximately 80 mL ultrapure water. The sample is then thoroughly mixed and 40 mL of sample are placed into a labeled sample bottle filled to zero headspace. Sample bottles are sealed and stored at 4°C until transfer to the NASA/MSFC Data Custodian.

6.10 Base Neutral and Acid Extractables, and Semi-Volatiles

Base/Neutral acid extractable quality control samples are prepared as a single sample. Each of the concentrate is equilibrated to ambient temperature. Preparation of these samples is accomplished by volumetrically pipeting 1.0 mL of each concentrate into a 1 L volumetric flask containing approximately 800 mL of ultrapure water. The concentrate must be added with the pipet tip held 1 cm below the surface of the water. Ultrapure water is added to the mark. Mixing is accomplished by inverting the flask several times. The entire 1 L volume is transferred into a labeled 1 L sample bottle. Sample bottles are sealed and stored a $4^{o}C$ until transfer to the NASA/MSFC Data Custodian.

7.0 SAMPLE TRANSPORTATION

QC Samples will be removed from the locked refrigerator and transferred to a locked cooler. The ACC, or his designee, will transport the QC samples to NASA/MSFC by automobile.

8.0 SAFETY

ERA samples are for laboratory use only. If any sample is ingested, notify the laboratory safety officer and contact a doctor immediately. Properly discard sample containers after use. For further information refer to individual Material Safety Data Sheets and/or the laboratory SOP document.

9.0 TRAINING

All technicians who prepare samples for this program will have at least 2 years of laboratory experience preparing reagent grade solutions. Technicians will work under the immediate supervision of the Analytical Control Coordinator until they demonstrate complete proficiency in the preparation of quality control samples.

BOEING LABORATORIES CHAIN OF CUSTODY RECORD

TYPE OF TEST:	SUBSYSTEM & LOCATION SAMPLED:	ANALYSIS REQUIRED
CUSTOMER:	CONTACT:	
BATCH # ________	PROTOCOL GROUP: ________ PARAMETER GROUP ________	

SAMPLE NO	SAMPLE DATE	SAMPLE TIME	SAMPLE *G	SAMPLE *W	FIELD TRACKING NUMBER	FIELD TRACKING NUMBER	NO. OF CONTAINERS	ANALYSIS REQUIRED	COMMENTS

1 SAMPLED BY & TITLE (SIGNATURE)	DATE/TIME	2 RELINQUISHED BY (SIGNATURE)	DATE/TIME	3 RECEIVED BY (SIGNATURE)	DATE/TIME

4 RELINQUISHED BY (SIGNATURE)	DATE/TIME	5 RECEIVED BY (SIGNATURE)	DATE/TIME	6 RELINQUISHED BY (Signature)	DATE/TIME	7 RECEIVED BY	DATE/TIME
8 RELINQUISHED BY (SIGNATURE)	DATE/TIME	9 RECEIVED BY MOBILE LAB FOR FIELD ANALYSIS	DATE/TIME	10 DISPATCHED BY	DATE/TIME	11 RECEIVED FOR LAB BY	DATE/TIME

ion

Figure 1 Chain of Custody

Appendix 3

Batch Designation Standard Operating Procedure

Batch Designation Standard Operating Procedure

Batch designations, like test schedules, will change from stage to stage. As a result, no specific batch designations can be noted here that will cover all tests to be conducted. A generalized procedure, however, has been developed. This procedure is followed for the assignment of batches for WRT Stages (beginning with stage 4*5).

1. Batch 1 is assigned to all fast turn-around ports (including safety samples). These samples are to be collected "as early as possible" on the scheduled test day, and every effort should be made to ship the samples to the laboratory before 10:00 am of the same day.

2. Batch 2 & 3 designations are assigned to the remaining ports involved in a test. The ports deemed of second priority to the fast turn-around samples are assigned a batch 2 designation. Every effort should be made to collect batch 2 samples before 2:00 pm. Lower priority ports are assigned the Batch 3 designation. Batch 3 samples should be shipped to the laboratory during late transportation runs (to be determined by the lead sampler).

3. Batch 4 is reserved for those samples which, for one reason or another, could not be collected or shipped to the laboratory during the ordinary working hours of a particular test day. These samples are assigned to batch 4 to avoid confusion with similar samples collected on the following test day.

4. Batch 5 is reserved for those samples which, due to the cycling of subsystems, cannot be scheduled for a particular collection time. These samples are collected as appropriate during the test day, and are shipped to the laboratory on the next available sample run following the sample collection.

5. Only the 5 batches noted above are utilized for ACC verified WRT stages. Additional batches may be added in the future as necessary.

The following is a list of the present batch collection priorities. This list is intended as a generalized rule, and specific incidents may require the collection of low priority batch samples before higher priority batch samples (such decisions should be coordinated between the Test Conductor, Data Custodian, and ACC).

Batch 1 > Batch 5 > Batch 2 > Batch 3 > Batch 4 as a general rule.
Batch 5 > Batch 1 > Batch 2 > Batch 3 > Batch 4 if the Batch 5 sample to be collected <u>must</u> be collected prior to the completion of Batch 1 collection (due to water availability, limited system cycling, etc.), or if the Batch 5 sample is a fast turn-around sample which does not fit the Batch 1 category due to its random nature.

Appendix 4

Sample Custody
Standard Operating Procedure

Sample Custody Standard Operating Procedure

The following sample custody Standard Operating Procedure (SOP), was developed as a modification to the custody procedure noted in the Analytical Control Test Plan (Version 3.0). This procedure will reduce sample handling, and help to reduce errors in shipping and custody.

1. Samples are collected by the Samplers[1] at the test site. The Lead Sampler[2] is responsible for collecting all samples as designated in the sample schedule. The sample schedule will be augmented by a Batch schedule denoting the time of day during which samples from particular sample ports should be collected. Additional samples from ports not noted on the Batch schedule, and those samples from ports which may only be collected during certain cycles of the test equipment, will be ordered by the Data Custodian and/or the NASA Test Director[3]. Since schedules are subject to change, the Data Custodian[4], Lead Sampler, and Test Director must stay in close communication with one-another to ensure that all of the test requirements are met with minimal difficulty.

2. After collection, the samples are placed in a box by the Sampler, along with complete (signed) and corresponding Chains of Custody. When all of the samples corresponding to the Chains of Custody are collected, the samples are delivered to (A) the Data Custodian's weighing table (when the Data Custodian is available to receive the samples), or (B) the samplers storage cooler (when the Data Custodian is not available to receive the samples). Should samples be delivered to the Data Custodian's table (as in step A), the Data Custodian assumes custody of the samples when he/she signs the corresponding Chains of Custody in the block entitled *Received By*. Should samples be delivered to the cooler (as in step B), the Data Custodian is responsible for taking the samples from the cooler, and takes custody of the samples when he/she signs the corresponding Chains of Custody in the block entitled *Received By*.

Boxes of samples should only be delivered to the cooler, or weighing table as complete sets. A sample set is defined as complete when the box containing the samples contains (A) all of the samples noted on the Chains of Custody found in the box <u>and</u> (B) all of the Chains of Custody corresponding to the samples found in the box. A complete set of samples can thus be comprised of one or many sample bottles.

When a Sampler signs a Chain of Custody in the block entitled *Relinquished By*, he/she is confirming that the Chain of Custody, and the associated samples in the box, comprise a complete set as defined above. When the Data Custodian signs a Chain of Custody in the block entitled *Received By* he/she is confirming the same. In addition, when the Data Custodian signs a Chain of Custody in the block entitled *Relinquished By* he/she is confirming that all of the samples noted on the Chain of Custody may be found in the cooler to which the Chain of Custody is attached. This signature also confirms that the samples being sent to the participating laboratory are only the samples assigned to that laboratory through scheduling, or schedule amendment.

3. After taking custody of the samples, the Data Custodian is responsible for weighing the samples, packing the samples in wet ice (in shipping coolers), and sealing the shipping coolers. The Chains of Custody corresponding to the samples in the shipping cooler should be placed in sealable plastic bags attached to the top of the shipping cooler. The shipping coolers should then be taped shut, and the Data Custodian must initial the tape across the point where the cooler top meets the body, on the front of the shipping cooler.

4. The Samplers are responsible for setting up transportation runs for the delivery of completed shipping coolers. These sample runs should follow the shipping schedule included in the batch schedule as closely as possible, but minor adjustments may be made to meet the requirements of each particular day. Such adjustments to the shipping schedule are the decision of the Lead Sampler, and should be coordinated with the Data Custodian and the Laboratory to ensure optimal utilization of the transportation service. The first two transportation runs of the morning are of particular importance (since they contain the fast turnaround samples), and every effort should be made to get these samples shipped on time.

5. The Lead Sampler, being responsible for communication with transportation, is also responsible for ensuring that all completed coolers are delivered to the laboratory. The Lead Sampler will know that a cooler is complete when it is sealed with tape, and initialed by the Data Custodian.

The Lead Sampler should maintain a log of transportation pickups. This log should include the time and date of the pickup, the signature of the transportation personnel providing the service, and the number of the cooler(s) picked up by transportation. All coolers should be marked with unique identification numbers to be determined by the Lead Sampler. When this procedure is followed, there is no need for the transportation personnel to sign individual Chains of Custody.

[1] Samplers are those personnel responsible for the collection of WRT samples, and work under the direction of a NASA Prime (this includes the Lead Sampler).

[2] The Lead Sampler or his/her designee.

[3] The NASA Test Director or his/her designee.

[4] The Data Custodian or his/her designee.

Appendix 5

On-Site Laboratory Monitoring
(Formal Laboratory Audits)

On-Site Laboratory Monitoring
(Formal Laboratory Audits)

The purpose of the site visit is to evaluate whether the testing laboratory is conducting its chemical and microbiological studies in compliance with the NASA/MSFC requirements. The list of questions in the following paragraphs is designed: (a) to provide an outline which can be used to collect important updated information on personnel, facilities, protocols, and record keeping as they relate to the MSFC specifications, and (b) to pinpoint specific problems and potential problem areas.

It should be emphasized that the inspector should use discretion to identify those areas in a particular facility that will need special attention. A careful check of these areas will be made, and if everything seems in compliance then he would proceed to the next step. But if problem areas or trouble spots are encountered then the inspector would investigate deeper. The questions raised in the following paragraphs are a comprehensive guideline of specific details. Not every item needs to be answered, and the question list provided should only be used as a guide. The specific study protocol, and, especially the SOPs would be source of checks for a specific study.

A. Administration

(1) Laboratory organization

- o Is the laboratory as organized by the institution adequate to ensure quality work performance?

(2) Management support

- o Does management adequately support the Laboratory Director and the Program?

(3) Support services

- o Are the support services required for the studies efficiently provided to the Laboratory Director? Are they under his/her direct control? If not, how are these coordinated?

(4) Quality Assurance Unit

(a) Is there a Quality Assurance (QA) unit that functions independently of the Testing Program, and reports directly to the Corporate Management?

(b) Who heads this unit? What are his/her qualification for this position? Examine his/her curriculum vitae.

(c) To whom does the Head of QA report?

(d) What is the personnel make-up of this unit? What are their qualifications?

(e) Do any of the QA personnel also function in any capacity in the Testing Program?

(f) If possible watch how a QA inspection is performed for a particular task. Do they use the SOPs to check that the steps are followed as documented?

(5) Master Schedule

(a) Does the Management (QA unit) maintain a Master Schedule for all testing activities at the facility?

The purpose of this activity is two-fold: First, the Master Schedule tells the inspector if the laboratory has possibly over-committed itself on tests and contracts with resulting degradation of performance. Second, the Master Schedule permits the ACC to schedule future facility inspection in a period of maximum interest and diverse activities.

(6) Standard Operating Procedures (SOPs)

The importance of SOPs, and the following of SOPs, during a study cannot be over emphasized. These are the principle organized documents to be checked during an inspection.

(a) Are there SOPs for all testing functions?

(b) Who writes them?

(c) Who reviews them?

(d) How often are they revised and updated?

(e) Are they available in the laboratory?

(7) Archives

(a) How are raw data handled and stored?

(b) Where are the archives located? In or out of the testing facility?

(c) Are supporting documents, stored in the same archival area or different?

(d) Is the organization of the archives adequate without being crowded and cluttered?

(e) Are the environmental conditions of the archival area adequate?

(f) What precautionary measures are installed against fire?

(g) Is the archival area secure with limited access?

(h) What are the procedures for removing material and returning these to the archives?

(8) Administration-Check list

(a) Have there been any changes in professional staff?

(b) Is the master schedule for all studies up to date? Is the facility over-committed?

(c) Is the Laboratory Director knowledgeable about the status of the following:

- o Chemicals?
- o Scheduling?
- o Record keeping?
- o Reporting?

(d) Has the Laboratory Director received and understood the following protocols:

- o Safety?
- o Chemical analyses, if performed by the testing laboratory?
- o Analytical controls?
- o Protocol documents?
- o Sampling?
- o What is the procedure for revision to the various protocols?

(e) Has the laboratory received all "Safety" packages related to use of individual chemicals required for Test?

(f) Are protocols available to appropriate staff? Protocols are available to appropriate staff members.

(g) Are there SOPs for all relevant areas of testing?

(h) Are all precautions (as necessary) in place before receipt of the chemical (e.g., handling, storage or disposal requirements)?

(i) How many types of accidents have been reported? What remedial action was taken?

(j) Have any personnel developed medical problems?

(k) Are personnel given regular medical check-ups? Who maintains these records?

(l) Where are the raw data archived?

(m) Who is the custodian of the archives?

(n) What is the condition of the archival area-environmental, organization, precaution against fire, security?

B. Chemistry

(1) Personnel

(a) What are the backgrounds of the responsible chemist and chemical support staff?

- o What is their experience in analytical chemistry or in performing analyses similar to those required in the program?

- o What experience do they have in analyzing and handling potentially hazardous or carcinogenic material?

(b) What are the backgrounds of the supervisor and technicians responsible for analytical analysis and interpretation?

(c) Is there a SOP for briefing technicians (i.e., a training program) on the proper handling of specific chemicals?

(2) Management

(a) What is the relationship of the analytical control group to the testing laboratory?

- o Is the analytical control group providing a service to the testing laboratory, or is it an integral part of the program?

(b) Is the responsible chemist knowledgeable of all the chemistry requirements of the testing?

(c) Has the responsible chemist discussed the scheduling of sample submissions with the Test Director, and is the schedule being met?

(d) Who is responsible for preparing reports on the analyses performed for the testing laboratory?

- o Are they reviewed for accuracy?

- o Who receives copies of these reports?

(e) What Quality Assurance (QA) procedures are followed by the analytical chemistry laboratory?

- Are SOPs available for laboratory activities?

(f) Have emergency procedures addressing such situations as chemical exposure or spills been established?

(3) Facilities and Equipment

(a) Analytical facility

- What analytical instrumentation is available for use in these studies?

- What is the maintenance schedule and quality assurance procedure used for each piece of equipment?

- Are the analytical laboratories designed and utilized in a safe and efficient manner?

- Can the facility handle potentially hazardous or carcinogenic materials?

- Is there adequate bench space to conduct the anticipated sample load?

- Is there adequate cold storage (at 5°C and -20°C or lower) for bulk chemical reference standards?

- How are wastes generated in the analytical facility disposed?

(4) Receipt, Storage, and Distribution of Bulk Test Chemicals

(a) Receipt

- Is there an SOP for receipt of bulk chemicals and transfer to the storage area?

- Was the chemical received in good condition?

- Was it properly packaged and labeled?

- Was a return receipt requested?
- Were the chemical reference standards for bulk chemical analyses pulled and stored at -20°C?

(b) Storage

- Was the material transferred directly from receipt to storage or was it repackaged?
- If repackaged, why and how was the repackaging done?
- Do storage conditions meet those recommended by the analytical laboratory or manufacturer?
- If multiple batches are received over the course of a study, are they used/analyzed on a first-in/first-out distribution system?
- Is special handling required and, if so, is the bulk broken down into working batches for easier handling?
- Are chemical reference standards stored at -20°C?

(c) Distribution

- Is material signed for when transferred for use?
- Is there an inventory system to keep track of amounts being used so that, if necessary, more can be ordered and received before a current batch runs out? Has consideration been given to storing a 90-day supply for emergency situations?
- Is material transferred from storage to mixing operations safely?
- When material is returned, is it sealed and stored properly?
- Are special handling conditions being followed by all personnel?

(5) <u>Analysis of Test Chemical</u>

Analysis of the test chemical may not be a requirement but it is performed the following items should be checked:

(a) Is the chemist provided with background information on chemicals, including an analysis report?

(b) Is the bulk material analyzed on a regular basis? What intervals are specified in the protocol? Are these being followed? Are the results consistent?

(c) Is the analysis protocol developed by the sponsor laboratory being followed? If not, why? If so, what approved modifications, if any, were necessary?

(d) Are the results reported to the Study Director? To sponsor?

(e) What QA procedures are used by the analytical laboratory for:

- o Calibration of instruments,
- o Running standard curves,
- o Use of internal standards,
- o Record keeping?

(6) <u>Preparation and Analysis of Standard Reference Material</u>

(a) Are standard mixtures analyzed promptly? Is there an adequate chain of custody procedure?

(b) Who performs the analysis?

- o If testing laboratory performs the analysis do they follow the method provided by the manufacturer? If not, why? If so, what modifications, if any, were necessary?

(c) What is the time frame between preparation and analysis?

(d) What is the QA on the analysis instruments used for standard analysis?

(e) How are the data monitored? How is the Laboratory Director informed if results are out of tolerance? What steps are taken to notify the proper personnel? What corrective actions are taken, and within what time frame?

(f) How are the data recorded, verified, and maintained? Are results within specified limits?

(7) Sample Receipt and Storage:

(a) Are chain of custody procedures followed?

(b) Are chain of custody forms maintained by laboratory?

(c) Are sample identification numbers maintained?

(d) Are new laboratory identification numbers assigned?

(e) Is a laboratory log of sample receipt maintained?

(f) Is the laboratory log computerized?

(g) Are the storage facilities adequate?

(h) Are storage facilities appropriate for specified preservation methods?

(i) Is the sample storage area secure from unauthorized entry?

(8) Sample Preparation and Extraction:

(a) Are separate preparation rooms utilized?

(b) Are decontamination procedures of preparation equipment appropriate for specified analyses?

(c) Is the frequency of decontamination appropriate?

(d) Are sample preparation techniques appropriate?

(e) Is a whole sample prepared and sub-sampled?

(f) Are extraction procedures, results, and dates documented?

(g) Are samples tracked through preparation and extraction?

(9) Sample Analysis:

(a) Do analytical methods comply with those specified?

(b) Is analytical equipment satisfactory?

(c) Is the analytical equipment under maintenance agreement?

(d) Is the equipment operated per manufacturer's instructions?

(e) Is the frequency of equipment calibration as specified by manufacturer and/or test documents more conservative?

(f) Are written calibration records including correlation coefficients maintained?

(g) Are written documentation on intermediate data (i.e. final volume of extract, dilution volumes, volume of sample injected, chromatograms, etc.) available for review by management or control personnel?

(10) Analytical quality control:

(a) Are spikes analyzed at least every 20 samples?

(b) Are frequency of split samples > 10 %?

(c) Is reagent blank frequency > 10 %?

(d) Is recovery compliance > 30 %?

(11) Data Reporting:

(a) Are written reporting of data results provided?

(b) Are laboratory QA/QC results with written report?

(c) Are method detection and quantification limits provided with the written reports?

(d) Is there provision for delivery of analytical results in digital format?

(12) <u>Sample Archiving and Disposal:</u>

(a) Are samples archived by laboratory?

(b) Is ultimate disposal of samples in compliance with sample contents?

(c) Are archiving facilities maintained separately from storage of un-analyzed samples?

(d) Are archiving periods as specified by the client followed?

Appendix 6

Preparationof Microbiological QA/QC Check Samples
Standard Operating Procedure

Preparationof Microbiological QA/QC Check Samples
Standard Operating Procedure

The following is a Standard Operating Procedure (SOP) for the preparation and submission of QA/QC samples for microbiological parameters. These samples will be used to assist the microbiological laboratory in determining possible sources of sample contamination, and as a source of quality assessment by the Analytical Control Coordinator (ACC). The samples to be submitted may be either sterile, or contaminated, but only sterile samples will be used for QA/QC purposes. This system will be implemented on a trial basis, and may be added to the AC Plan, and FEDS at a later date.

1. All bottles used will be standard WRT test micro bottles (polypropylene). The bottles will be cleaned with hot soapy water, and rinsed at least 10 times with ultrapure water prior to sterilization.

2. Sample Collection- Samples will be collected from high-purity water at a purity of 17.9 Mohm-cm or higher. The standard preservatives used for WRT micro samples will be added, and the water will be sterilized as in **step 3**.

3. Sample Sterilization- Samples will be autoclaved at 121°C for a minimum of 30 minutes. A log of the autoclave times and temperatures will be maintained.

4. Sample Distribution- Samples will be distributed along with the regular microbiological samples during testing. The samples will be labeled to match the scheme of the test stage being conducted, and will utilize the AC ports 39 and 98.

5. Result Reporting- Sample results should be reported to the ACC by FAX through the laboratory QA/QC department, or data manager. Results will be expected by the ACC within 24 hours of the analysis time; any delays should be reported by telephone to the ACC.

Appendix 7

ACC's Data Review
Standard Operating Procedure

ACC's Data Review
Standard Operating Procedure

Due to the slow turn-around of analytical data by the FEDS relative to the pace of a normal WRT stage, the ACC has adopted a method of data review which does not utilize the FEDS. Under this system of hard copy review, all AC sample results are transmitted two or more times per day, by FAX, to the ACC from the participating laboratories. These results are compiled by the ACC, and compared to the certified values of the control samples submitted. Acceptable and Suspect ratings are assigned by the ACC, and the results are reported to the QC Coordinator of the laboratory in question. The results are reported by the ACC within 4 hours of receipt, or the following morning when received after 1:00 pm. All Suspect sample ratings are accompanied by the complete NASA sample number, the laboratory sample result, and the Minimum and Maximum values assigned to the certified sample. These results are reported by telephone to the QC Coordinator, but may be transmitted by FAX at his or her request.

Appendix 8

New Stage Designation
Standard Operating Procedure

New Stage Designation Standard Operating Procedure

Stages are assigned during WRT work to designate different data groups for the purposes of data basing and data evaluation. Due to the complexity of the WRT, it is difficult to design a system which will cover all of the possible scenarios which may develop. A variety of developments may require data to be separated from other larger groups of data, and the judgement of the test team should be used in determining the switch to a new stage. As a base rule, however, test stages should remain constant as long as the water being used for the test(s) involved is the same. A note of the special nature of the data can be logged in the data base based on the Stage day. Such a system gives the person evaluating the data a general idea of the "age" of the water being utilized in the test.

When new stages are to be designated, the information flow figure entitled Test Changes (Appendix 9) should be utilized. Such procedure will help to ensure that all those involved in WRT activity are aware of the changes in testing.

Appendix 9

Chains of Communication

Chains of Communication

For the most part, it is impossible to predict the problems which may occur during WRT activities. Since problems do arise however, it is essential that information flows to all those individuals responsible for activities pertaining to the test. The following figures have been developed to assist everyone in passing information along.

FIGURE 1

The Medical Alert Notification Procedure shows the flow of information necessary during medical safety alerts. The block in the upper left corner of the diagram encloses the "Normal Notification Procedure". This flow of information takes place on a daily basis during testing, and is necessary for the continuation of testing. During medical emergencies, however, other notification procedures are necessary.

Scenario 1

When a possible medical emergency is identified by the participating laboratory. The Data Custodian and EL65, through the test director, are responsible for getting medical information to ED62 (Test coordinator or his/her designee) so that the information may be distributed to the medical monitor and the rest of the WRT team as shown in the diagram. Once such information is received within an organization, the individual receiving the information is responsible for passing the word on to other team members within the organization, and to all subcontractors requiring the information.

Scenario 2

When a possible medical emergency is identified by the Medical Monitor (through physical exam, etc.), the information is relayed to ED62 (Test coordinator or his/her designee). ED62 is then responsible for passing the information on to all members of the WRT team (including, in this instance, the Data Custodian, and EL65). Those individuals within each organization receiving the information are responsible for ensuring that the information is passed on to all other team members within the organization, and to all subcontractors requiring the information.

Scenario 3

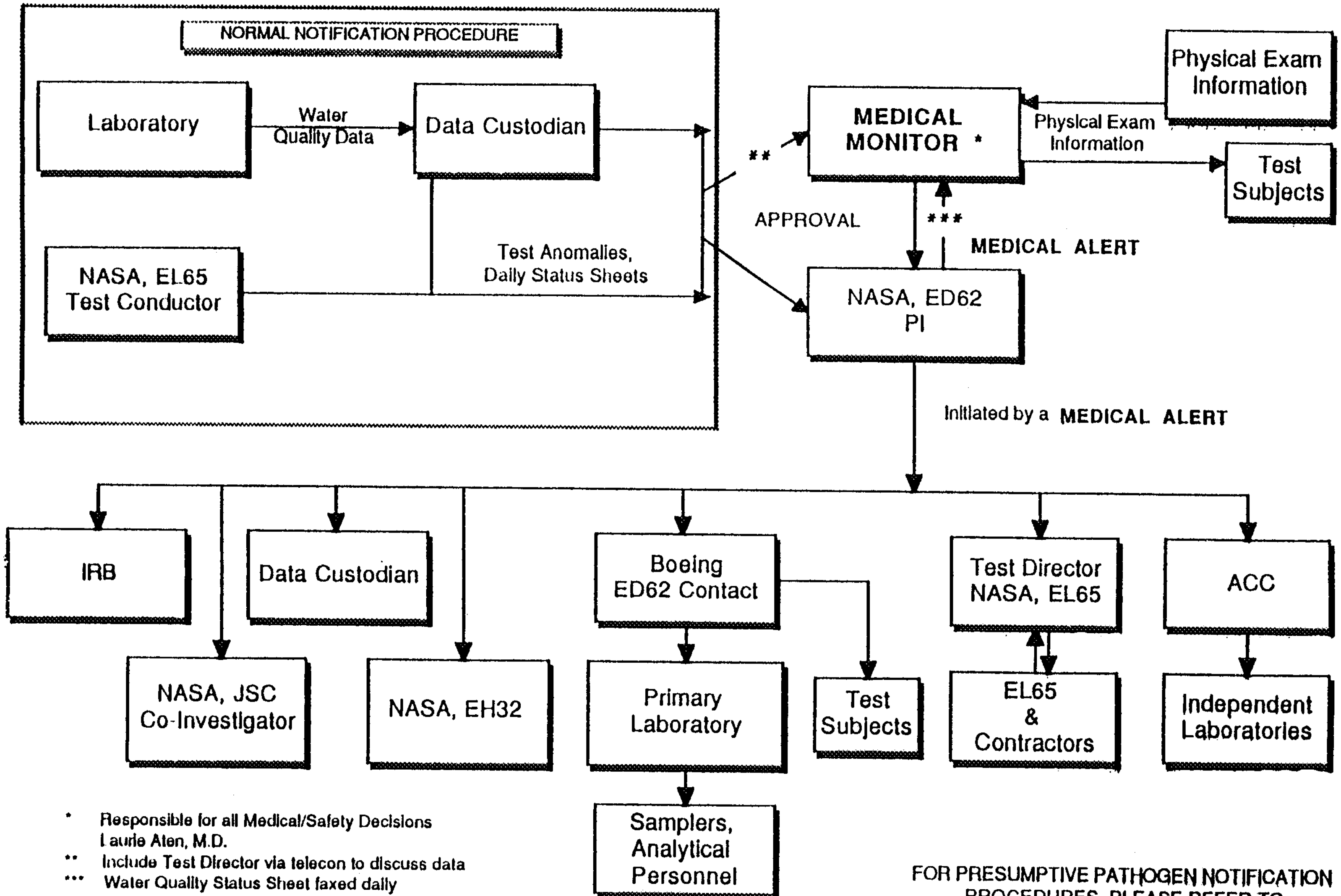
When a possible medical emergency is identified by the Data Custodian, or EL65, the information will be passed on to ED62 (Test coordinator or his/her designee) and then handled as in scenario 1.

FIGURE 2

Methods, Preservatives, Detection Limits, and Units, due to changes in technology, and/or laboratory capability, may change. When such changes are required, certain individuals involved with the WRT must know of the change in order to properly update records, manuals, data bases, etc. Such changes should be coordinated by those individuals listed in the upper portion of the diagram. Once decided, the information is passed on to ED62 by the Prime's QA/QC Coordinator. The information flows that follow are self explanatory.

FIGURE 3

Changes to testing including sample schedule changes, chain of custody errors/changes, and test delays are among the most commonly occuring modifications, and effect the greatest number of team members. This being the case, the flow of information, and coordination of changes between NASA branches, is extremely important. The upper portion of Figure 3 shows the coordination of test changes through the development of a Test Preparation Sheet (TPS). Once the decision is made to implement a TPS, the information contained in the document is passed on to the NASA prime contractors by ED62 (Test coordinator or his/her designee), and to the Data Custodian team by EL65. The individual contacted within each organization is responsible for informing others in the organization of the change.



* Responsible for all Medical/Safety Decisions
Laurie Aten, M.D.
** Include Test Director via telecon to discuss data
*** Water Quality Status Sheet faxed daily

Principal Investigator (PI)
Analytical Control Coordinator (ACC)
Institutional Review Board (IRB)

FOR PRESUMPTIVE PATHOGEN NOTIFICATION PROCEDURES, PLEASE REFER TO FIGURES 6-7 AND 6-8

FIGURE 1 MEDICAL ALERT NOTIFICATION PROCEDURE

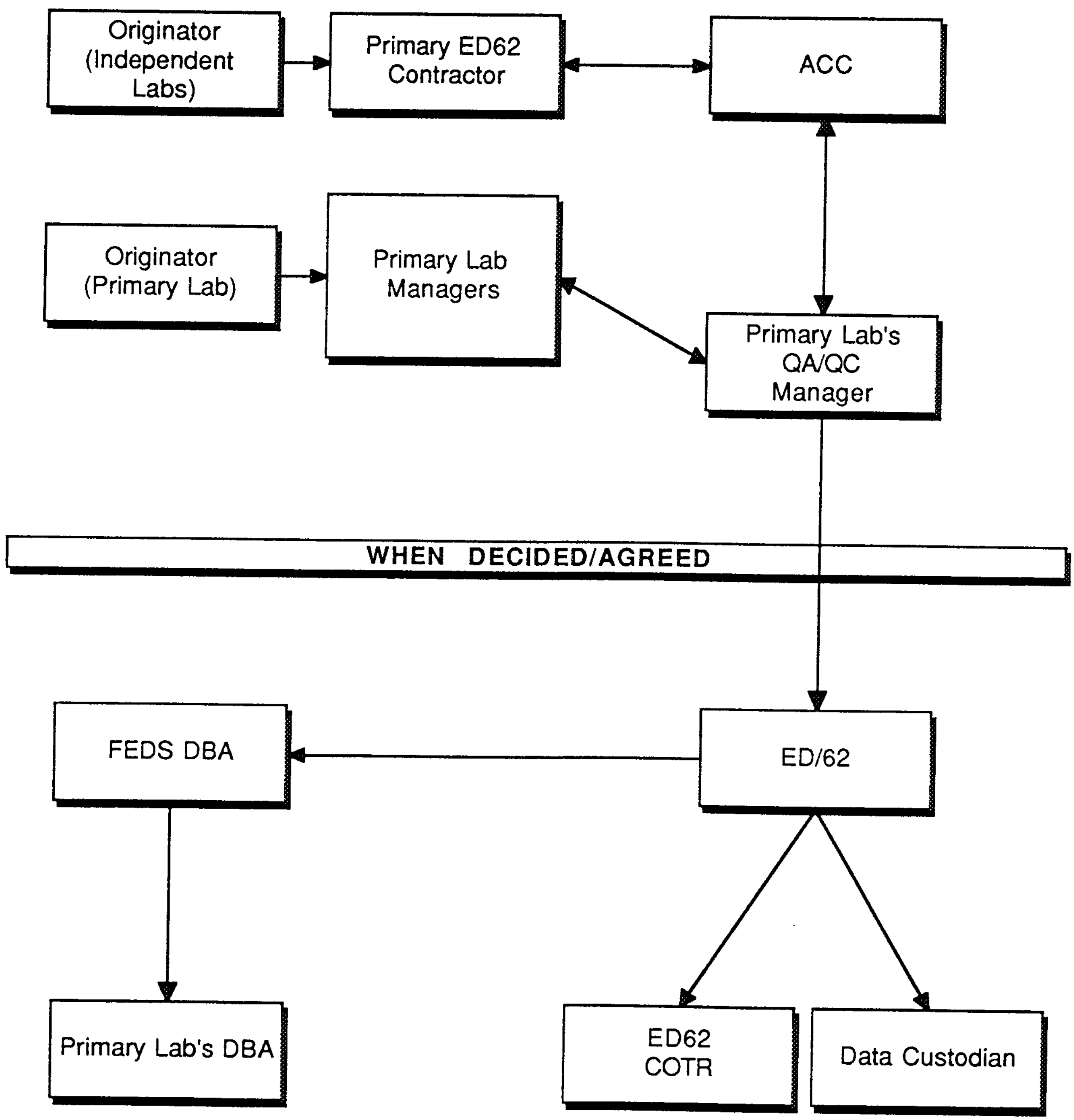
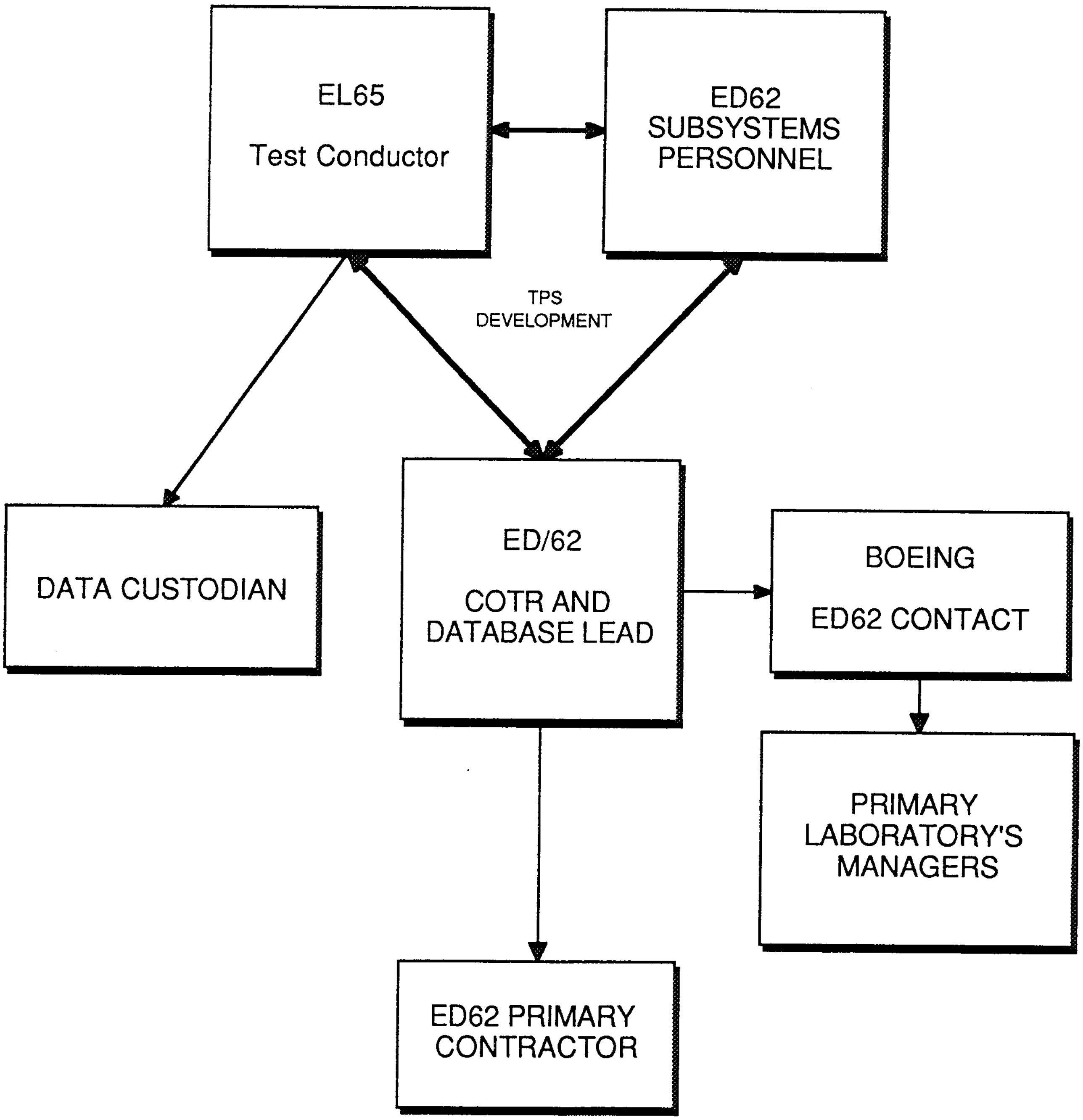


FIGURE 2 CHANGE NOTIFICATION PROCEDURE



THOSE INDIVIDUALS LISTED ARE RESPONSIBLE FOR INFORMING OTHERS IN THEIR ORGANIZATION OF THE CHANGE IN TESTING

FIGURE 3 TEST CHANGES

Appendix 10

Microbiological Enumeration
Methods

ENUMERATION OF AEROTOLERANT HETEROTROPHIC BACTERIA USING R2A AGAR

This procedure is designed for the isolation and enumeration of heterorthophic bacteria from water samples containing low concentrations of organic carbon. The medium used, R2A, is a low nutrient medium designed to culture heterotrophic bacteria. This includes oligotrophic bacteria characteristically found in aquatic environments.

Media Preparation. Suspend 18.2 grams of Difco R2A medium in 1 liter of deionized/distilled water, using a 2-liter flask. Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat. The final pH of the medium should be 7.2 at 25°C.

Sterilization. Autoclave at 121°C for 35 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Dispensing. Aseptically dispense the sterile medium (7 ml) into sterile 50 x 9 mm Petri dishes with tight fitting lids and 100 x 15 mm standard petri dishes.

Quality control. The prepared medium should be light amber in color and translucent. Darkened medium may indicate an increased time or temperature in sterilization. Allow the agar to solidify and cool and then incubate at room temperature for 48 hours to permit adequate drying and as a check for contamination. Record the number of contaminated plated in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Positive control for media preparation. Prepare a positive control using *Staphylococcus epidermidis* (ATCC 12228). The resultant colonies should appear small, round and smooth with a creamy white color. This is not a selective medium, therefore no negative control is needed.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Processing dirty samples. Serially dilute (6 tubes) and spreadplate the last four dilutions.

ENUMERATION OF AEROTOLERANT HETEROTROPHIC BACTERIA USING R2A AGAR (cont.)

__Processing clean samples__. Follow the Membrane Filtration Method described in the section on MEMBRANE FILTRATION. Aliquots of 100, 10, and 1 ml are to be filtered. Aseptically transfer the filters to plates.

__Incubation__. Incubate the cultures aerobically at 30^{o}C and examine the plates after 2 and 5 days. Count the colonies under the stereomicroscope (10 x 15X) at the times of each observation, marking the position of each with a Sanford's Sharpie. Colonies that become apparent on subsequent observations should be added to the total count.

__Interpretation of results__. There may be a variety of colony morphologies and pigmentation as this procedure will culture many bacterial types. Count all colonies present. The countable range of colonies is between 20-60 colonies per plate on small plates and 30-300 colonies on the large plates.

__Confirmation__. All colony types will be identified.

ENUMERATION OF ANAEROBIC BACTERIA

This procedure is designed for enumeration of anaerobic bacteria from WRT water samples. The key to success in culturing anaerobes is to maintain anaerobic conditions. This is accomplished by: 1) removing oxygen from the medium by boiling, 2) incorporating reducing agents in the media and dilution buffers and 3) working in an oxygen-free atmosphere (anaerobic chamber).

Media Preparation. Boil a liter of deionized or distilled water for 5 minutes to remove oxygen and then add 58 grams of Difco Brewer Anaerobic Agar (Catalog No. 0279-01-3). Continue boiling to dissolve completely, then promptly remove the medium from the heat. The final pH should be 7.2 at 25°C.

Sterilization. Autoclave at 15 lbs pressure for 30 minutes (121°C). Cool the medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 15 minutes between preparation and autoclaving. Do not allow the medium to remain at 50°C for more than one hour before pouring plates.

Dispensing. Aseptically dispense the sterile medium into sterile 100 x 15 mm Petri dishes. Allow the agar to solidify and then transfer to an anaerobic chamber. Allow the plates to incubate for 48 hours before use. Poured plates must be stored immediately upon solidification for 1-2 days in an oxygen-free atmosphere such as the Gaspak 150 Anaerobic System (Catalog No. J3051-5, Baxter Scientific) prior to use.

Quality Control. The prepared medium should appear beige. A red color indicated the presence of oxygen. Do not use any plates that have turned red. Allow plates to incubate for 24 hours under anaerobic conditions and inspect for contamination. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% are contaminated, discard the entire batch. Inoculate a petri plate with *Clostridium beijerinckii* (ATCC 17795). The resultant colonies should be circular to irregular, entire to scalloped, flat to raised, translucent, grey in color, shiny and smooth. As a negative control, inoculate a second plate with *Xanthomonas maltophilia* (ATCC 13637). There should be little or no growth on this plate. Record the results obtained for each batch in the Media Preparation Log Book.

Storage. The prepared plates must be stored at 4°C in an anaerobic jar and used within 28 days. Plates should be stored in the dark and allowed to come to room temperature under anaerobic conditions just prior to use.

ENUMERATION OF ANAEROBIC BACTERIA (cont.)

Dirty samples. All work is to be conducted under an oxygen-free atmosphere (Anaerobic Chamber). Serially dilute (6 tubes) water samples using Brewer thioglycollate medium (Difco 0236-01-5). A blue color in this medium indicate the presence of oxygen. Plate 0.1 mL of each dilution onto Brewer Anaerobic Agar and incubate in a Gas pac. Examine after five days. Enumerate each colony type and restreak a representative of each colony type onto Brewer Anaerobic Agar to obtain pure cultures. Incubate in a Gaspak and examine after 5 days. Restreak onto two plates. Incubate one plate aerobically and the other anaerobically. Identify all cultures that grow anaerobically, but not aerobically.

Clean samples. All work is to be conducted under an oxygen-free atmosphere (Anaerobic Chamber). Fill a syringe with inert gas (N_2), then empty it by pushing in the plunger. Draw the sample into the syringe, remove the needle and replace it with a Swinnex Disc filter holder (Millipore SXOO O47OO) containing a 47 mm, 0.45µM filter. Filter the sample by attaching a vacuum to the Swinnex. Remove the vacuum line and replace the syringe with a second syringe that has been filled with inert gas. Draw the gas through the filter. Immediately transfer the filter to a pre-reduced agar plate and incubate under anaerobic conditions (Gas pac). Samples of 50, 5, and 1 mL are to be filtered. Sample dilutions of 0.1, 0.01, and 0.001 mL are to be prepared using Brewer thioglycollate medium (Difco 0236-01-5). A blue color in this medium indicates the presence of oxygen.

Incubation. Incubate the cultures anaerobically at 30°C and examine after 48 hours and 5 days. The anaerobic environment can be produced by the use of a commercially available anaerobic system consisting of an anaerobe jar, gas packs and palladium catalyst. The use of an anaerobic test strip is required to insure achievement of an anaerobic environment.

Interpretation of results. This method will allow the recovery of both anaerobic and facultatively anaerobic bacteria. Examine the plates after 48 hours. The countable range of colonies with this medium is between 30-300 colonies per plate.

Confirmation. All isolates must confirmed as anaerobes. Plate each isolate onto Brewer's anaerobic agar and incubate aerobically. Strict anaerobes will not grow under aerobic conditions. Positively confirmed anaerobes will be identified.

ENUMERATION OF POTENTIAL PATHOGENS (MYCOBACTERIUM)

Nineteen different mycobacterial species are associated with disease in humans. Diseases range form superficial infections to invasion of the lungs and other internal organs. Mycobacteria can also occur in soil and water where they can be a potential danger. Middlebrook media are commonly used for isolation and cultivation of mycobacteria. Although the presence of malachite green makes the medium slightly selective, the medium is formulated primarily to permit growth of fastidious mycobacteria.

Preparation Mycobacterium agar (MMA). Suspended 21 grams of Bacto Mycobacterium 7H11 agar medium and 5.0 mL glycerol in 900 mL of deionized/distilled water, using a 2-liter flask. Heat with continuous stirring allowing the medium to boil in order to completely dissolve the agar, then promptly remove the medium from the heat.

Sterilization. Autoclave at 121^{o}C for 35 minutes. Cool medium to 50^{o}C in a preheated water bath.

Addition of enrichment. Add 100 mL of Bacto Middlebrook OADC Enrichment w/WR 1339 and mix thoroughly.

Dispensing. Aseptically dispense the sterile medium into sterile 100 x 15 mm standard petri dishes.

Processing dirty samples. Serially dilute (6 tubes) and spreadplate. After 2 and 10 days, count colonies, and transfer colonies to fresh plate to obtain pure cultures for identification.

Processing clean samples. Follow the Membrane Filtration Method described in the section on MEMBRANE FILTRATION (SOP p. 24). Aliquots of 100 and 10 mL are to be filtered. Aseptically transfer the filters to plates. After 2 and 10 days, count colonies and transfer colonies to fresh plate to obtain pure cultures for identification.

Quality control. Allow the agar to solidify and cool and then incubate at room temperature for 48 hours to permit adequate drying and as a check for contamination. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch. The dehyrated medium is light beige with a slight greenish tint. The prepared medium is light amber to grey. The Middlebrook enrichment liquid is light amber.

ENUMERATION OF POTENTIAL PATHOGENS (MYCOBACTERIUM) (cont)

Positive control. Streak a plate with *Mycobacterium fortuitum*, ATCC 6841. Good growth should occur within four days.

Negative control. Since this is not a selective medium, there is no negative control.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Incubation. Incubate aerobically at 30°C and examine after 2 and 10 days. Count the number of colonies of each colony type and restreak a representative colony of each type for identification.

Confirmation. All colony types yielding Gram positive rods or filaments will be identified.

REFERENCES:

Linette, E.H., A. Balows, W.J. Hausler and H.J. Shadomy. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, DC. pp. 216-248.

Difco Manual, 10th ed. 1984. pp. 567-569.

ENUMERATION OF POTENTIAL PATHOGENS (FECAL STREPTOCOCCI/ENTEROCOCCI)

Fecal streptococci include S. faecalis, S. faceum, S. avium, S. bovis, S. equinus, and S. gallinarum. The normal habitat of these organisms is the intestinal tract of warm blooded animals. Members of the antigenic Group D (S. faecalis, S. faceum, S. avium, S. gallinarum) are called enterococci and have been used as indicators of the extent of fecal contamination of water. Do not overheat. mE agar is highly selective for fecal streptococci.

Preparation of mE media. Suspend 42 grams of Bacto m Enterococcus Agar in 1 liter of deionized/distilled water, using a 2-liter flask. Heat with continuous stirring allowing the medium to boil in order to completely dissolve the agar, then promptly remove the medium from the heat.

Sterilization. Autoclave at 121°C for 35 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Dispensing. Aseptically dispense the sterile medium (7 ml) into sterile 50 x 9 mm Petri dishes with tight fitting lids and 100 x 15 mm standard petri dishes.

Processing dirty samples. Serially dilute (6 tubes) and spreadplate. After 48 hours, count colonies and transfer colonies to fresh plate to obtain pure cultures for identification.

Processing clean samples. Follow the Membrane Filtration Method described in the section on MEMBRANE FILTRATION (SOP p.24). Aliquots of 100, 10, and 1 ml are to be filtered. Aseptically transfer the filters to plates. After 48 hours, count colonies and transfer colonies to fresh plate to obtain pure cultures for identification.

Quality Control. The dehydrated powder should be light beige and the prepared medium should be light amber. Allow the agar to solidify and cool and then incubate at room temperature for 48 hours to permit adequate drying and as a check for contamination. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Positive control. Streak a plate with Streptococcus faecalis ATCC 19433 or 29212. There should be good growth in 24 hours and the colonies should be pink to red.

ENUMERATION OF POTENTIAL PATHOGENS (FECAL STREPTOCOCCI/ENTEROCOCCI) (cont)

Negative control. Streak a plate with *Escherichia coli* ATCC 25922. There should be no growth after 24-48 hours.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Incubation. Incubation aerobically at 35°C and examine after 48 hours. Count the number of colonies of each colony type and restreak a representative colony of each type for identification.

Interpretation of results. Enterococci form pink to dark red colonies, 0.5-3 mm in diameter.

Confirmation. All pink or red colony types are suspected fecal streptococci. However, all colony types will be identified because this medium has also been shown to support the growth of catalase positive, gram positive cocci.

REFERENCE:

Linette, E.H., A. Balows, W.J. Hausler and H.J. Shadomy. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington DC. pp. 154-170.

Difco Manual, 10th ed. 1984. pp. 346-348.

ENUMERATION OF STALKED BACTERIA AND OTHER OLIGOTROPHS

Although Caulobacter is ubiquitous in soil and aquatic environments, it does not compete well with faster-growing organisms such as pseudomonads in rich nutrient media. Consequently, its presence can be overlooked during routine culturing of water samples. Growth of Caulobacter and other organisms that prefer a low-nutrient environment can eventually clog filters and cause buildup of biomass on pipes. Since the water recycle system will function for long periods of time with continuous water flow, it is of interest to determine whether these organisms are present.

Preparation of enrichment medium. Suspend 0.1 grams of Bacto Peptone in 1 liter of tap water, using a 2-liter flask. Dispense 50 mL portions in 250 mL flasks. Autoclave immediately. If 250 mL flasks are not available, large test tubes, half-filled with medium can be used.

Preparation of Caulobacter Isolation Medium. Suspend 1.0 gram Bacto Peptone, 0.5 grams Yeast Extract, 0.1 gram $MgSO_4$ - $7H_2O$ and 10 grams Agar in 1 liter of distilled/deionized water. Heat with continuous stirring allowing the medium to boil in order to completely dissolve the agar, then promptly remove the medium from the heat.

1% Peptone. Add 1.0 gram Bacto Peptone to 100 mL tap water and autoclave.

Sterilization. Autoclave at 121°C for 35 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Dispensing. Aseptically dispense the sterile medium into sterile 100 x 15 mm standard petri dishes.

Processing dirty samples. Add 1 mL of dirty sample water per 50 mL of enrichment medium and incubate at room temperature for one to three weeks. Examine for presence of pellicle. Remove portion of the pellicle and examine under the microscope for presence of stalked bacteria. If present, streak pellicle onto Caulobacter Isolation Medium. Examine Isolation Medium under a stereomicroscope after one week and identify Caulobacter by microscope observation of wet mounts.

ENUMERATION OF STALKED BACTERIA AND OTHER OLIGOTROPHS (cont)

Processing clean samples. Add 0.1 mL of 1% peptone solution per 10 mL of clean water sample in a large sterile test tube and incubate at room temperature for one to three weeks. Examine for presence of pellicle. Remove portion of the pellicle and examine under the microscope for presence of stalked bacteria. If present, streak pellicle onto Caulobacter Isolation Medium. Examine Isolation Medium under a stereomicroscope after one week and identify Caulobacter by microscopic observation of wet mounts.

Positive control. Inoculate liquid medium with stock culture of Caulobacter. Process same as regular samples.

Negative control. Incubate uninoculated liquid medium and process same as regular samples.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Incubation. Incubate liquid cultures at room temperature without shaking and examine after one week. Examine under the microscope for presence of stalked bacteria.

Interpretation of results. Caulobacter are identified by the presence of stalks. They are difficult to see, even with phase contrast.

Confirmation. Microscopic observation confirms the presence of Caulobacter.

REFERENCE:

Gerhardt, P. 1981. Manual of Methods for General Microbiology. American Society for Microbiology, Washington, DC. p. 122.

Aaronson, S. 1970. Experimental Microbial Ecology. Academic Press. New York. p. 129.

ENUMERATION OF THIOBACILLUS (CORROSION)

Iron and sulfur oxidizing bacteria belonging to the genus Thiobacillus, contribute to corrosion in water systems. They are strict aerobes that use carbon dioxide as a carbon and reduced sulfur compounds or elemental sulfur as an energy source. The sulfuric acid produced can contribute to corrosion. Some thiobacilli can drive energy by oxidizing ferrous to ferric, thus participating directly in the corrosion process.

T. thioparus, *T. denitrificans*, *T. novellus*, and *T. intermedius* can use thiosulfate as an energy source and can grow at pH 7 whereas *T. thiooxidans* and *T. ferrooxidans* use elemental sulfur, but not thiosulfate and grow in the pH range below 4.5. Therefore two media types are required for detecting the presence of thiobacilli in water samples.

Preparation of acidic Thiobacillus Broth or Agar (ATA). Suspend 0.08g chlorophenol red, 10 g sodium thiosulfate $-5H_2O$, 1.0 g ammonium chloride, 0.5 g magnesium chloride, 0.6 g K_2HPO_4, 0.4 g KH_2PO_4, 0.02 g $FeCl_3$, and 0.05 g yeast extract in 1 liter of distilled/deionized water. Adjust pH to 4.5 with 0.1N H_2SO_4. Add 0.1N H_2SO_4 a drop at a time. Allow pH to stabilize (15 sec) before adding the next drop. For solid media add 15 grams Agar after adjusting the pH.

Preparation of neutral Thiobacillus Agar (NTA). Same medium as above, adjusted to pH 7.0.

Preparation of Waksman and Joffe liquid flower sulfur medium (LTA). Suspend 3.5 g K_2HPO_4, 0.3 g ammonium sulfate, 0.5 g magnesium sulfate.$7H_2O$, 0.02 ferrous sulfate.$7H_2O$, and 0.25 g calcium chloride in 1 liter of distilled/deionized water. Adjust pH to 4.5 with 0.1N sulfuric acid. Dispense 10mL of medium into each test tube. Add powdered elemental sulfur (0.05g/10mL to each tube and sterilize at 100^{o}C for 30 min for three days.

Sterilization. Sterilize Waksman and Joffe medium at 100^{o}C for 30 min for three days. Autoclave agar media at 121^{o}C for 35 minutes. Cool medium to 50^{o}C in a preheated water bath.

Dispensing. Aseptically dispense the sterile medium into sterile 100 x 15 mm standard petri dishes.

Quality control. Inoculate media with *T. thiooxidans* and *T. intermedius*. *T. thiooxidans* should grow in the acid medium with sulfur, but not on the medium containing thiosulfate. *T. intermedius* should grow in the thiosulfate, but not elemental sulfur.

ENUMERATION OF THIOBACILLUS (CORROSION) (cont)

Storage. Media may be stored at 4°C for 30 days and used within 30 days.

Processing dirty samples. Serially dilute the water sample (3 dilution tubes) and spreadplate onto ATA and NTA. Incubate at room temperature and observe after 5, 10 and 20 days with a stereomicroscope. Transfer 1 mL of each dilution into 10 mL of the liquid thiobacillus medium (LTA).

Processing clean samples. Filter 10 mL of the water sample onto a 0.45µ filter and place the filter in a test tube containing liquid thiobacillus medium (LTA). Also filter 10 mL portions of the water sample and transfer the filter to the surfaces of ATA and NTA.

Incubation. Incubate the cultures at room temperature and examined after 2 and 7 days. Count colonies on agar plates and record lowest dilution yielding growth in LTA.

Interpretation of results. Presence of thiobacilli will be indicated by growth of small colonies on agar media and slight increase in turbidity of the liquid medium.

Confirmation. Streak colonies for isolation of Thiobacillus agar without yeast extract and on TSA. Incubate at room temperature for 2 days. Growth on Thiobacillus agar with yeast extract but not TSA indicates the presence of *Thiobacillus* species.

REFERENCES:

Standard Methods for the Examination of Water and Wastewater 17th ed. 1989. American Public Health Association, Washington DC. pp. 9-114 to 9-126.

Aaronson, S. 1970. Experimental Microbial Ecology. Academic Press, New York. pp. 116-119.

ENUMERATION OF ACTINOMYCETES

The actinomycetes and related organisms are a broad group of bacteria that are involved in degradation of organic compounds. They are widely distributed in soil and water and contribute to slime formation and to odor problems through geosmin production.

Preparation of Starch-casein agar (SCA). Suspend 10 grams of soluble starch, 0.3 grams casein (not casein hydrolysate or casitone), 0.05 grams cycloheximide, 2.0 grams potassium nitrate, 2.0 grams sodium chloride, 2.0 grams K_2HPO_4, 0.05 grams magnesium sulfate heptahydrate, 0.02 grams calcium carbonate, 0.01 grams ferrous sulfate heptahydrate, and 15 grams agar in 1 liter of deionized/distilled water, using a 2-liter flask. Heat with continuous stirring allowing the medium to boil to completely dissolved the agar, then promptly remove the medium from the heat.

Preparation of Glycerol arginine agar. Suspend 20 mL glycerol, 1.0 gram arginine, 0.05 grams cycloheximide, 2.0 grams potassium nitrate, 2.0 grams sodium chloride, 2.0 grams K_2HPO_4, 0.05 grams magnesium sulfate heptahydrate, 0.02 grams calcium carbonate, 0.01 grams ferrous sulfate heptahydrate and 15 grams agar in 1 liter of deionized/distilled water, using a 2-liter flask. Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat.

Sterilization. Autoclave at 121^{o}C for 35 minutes. Cool medium to 50^{o}C in a preheated water bath.

Dispensing. Aseptically dispense the sterile medium into sterile 100 x 15 mm standard petri dishes.

Processing dirty samples. Serially dilute (6 tubes) and spreadplate. After 2 and 10 days, count colonies and transfer colonies to fresh plates to obtain pure cultures for identification.

Processing clean samples. Follow the Membrane Filtration Method described in the section on MEMBRANE FILTRATION. Aliquots of 100 and 10 mL are to be filtered. Aseptically transfer the filters to plates. After 2 and 10 days, count colonies and transfer colonies to fresh plates to obtain pure cultures for identification.

Quality control. Allow the agar to solidify and cool and then incubate at room temperature for 48 hours to permit adequate drying and as a check for contamination. Record all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

ENUMERATION OF ACTINOMYCETES (cont)

Positive control. Streak a plate with Streptomyces spp. Good growth should occur within four days.

Negative control. Since this is not a selective medium, there is no negative control.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Incubation. Incubate aerobically at 30°C and examine after 2 and 10 days. Count the number of colonies of each colony type and restreak a representative colony of each type for identification.

Confirmation. All colony types yielding Gram positive rods or filaments will be identified.

REFERENCES:

Standard Methods for the Examination of Water and Wastewater 17th ed. 1989. American Public Health Association, Washington, DC. pp. 9-126 to 9-129.

Aaronson, S. 1970. Experimental Microbial Ecology. Academic Press, New York. pp. 135-146.

ENUMERATION OF THERMOPHILES

Thermophiles are microorganisms that can grow at temperatures above 45-50°C. Since thermophiles are physiologically diverse (autotrophs and heterotrophs), no one medium will culture all thermophiles. The procedure described below should be the least selective for heterotrophic thermophiles.

Enrichment procedure for dirty samples. Transfer 1 to 10 mL of the water sample to 40 mL trypticase soy broth in a 250 mL flask. The amount added depends on the turbidity of the water sample. The final suspension in trypticase soy broth should be no more than barely turbid. Record amount of water sample added. Incubate at 55°C in a shaking water bath (200 rpm) and observe for increase in turbidity aft 1, 2, and 3 days. As soon as a significant increase in turbidity occurs, streak onto preheated (55°C) trypticase soy agar and incubate at 55°C. Reculture the predominant colony types and identify.

Direct plating for dirty samples. Prepare a serial dilution of the water sample and spread-plate 0.1 mL onto trypticase soy agar pre-equilibrated at 55°C. Incubate for 24-48 hours and record number of colonies. Restreak predominant colony types on trypticase soy agar, incubate at 55°C for 24 hours and identify.

Enrichment procedure for clean samples. Filter 10 to 100 mL of the water sample onto a membrane filter as previously described. Aseptically transfer the filter to a 250 mL flask containing 40 mL trypticase soy broth. Record amount of water sample filtered. Incubate at 55°C in a shaking water bath (200 rpm) and observe for increase in turbidity aft 1, 2, and 3 days. As soon as a significant increase in turbidity occurs, streak onto preheated (55°C) trypticase soy agar and incubate at 55°C. After 24 hours, restreak predominant colony types and identify.

Preparation of media. For liquid media, suspend 30 grams of Difco trypticase soy broth in 1 liter of distilled, deionized water. Transfer 40 mL to 250 mL flasks and autoclave for 15 minutes.

For solid media, suspend 30 grams of Difco trypticase soy broth and 15 grams Bacto agar in 1 liter of distilled, deionized water in a 2-liter flask and autoclave for 40 minutes. Allow media to cool to 50°C in a water bath and aseptically dispense into standard (100 x 15 mm) petri dishes. Incubate plates at 55°C for 24 hours before use to reduce excess moisture.

Quality control. The medium should appear light amber in color and translucent. A darkened medium may indicated an increased time or temperature in sterilization or charring during initial heating. Allow plates to incubate at 55°C, for 24 hours and inspect for contamination prior to usage.

ENUMERATION OF THERMOPHILES (cont)

Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10%of the plates are contaminated, discard the entire batch. Record temperature of 55 C water bath and 55 C incubator daily. Maintain water supply in bottom of 55 C incubator.

Positive control. Prepare a positive control using *Bacillus stearothermophilus* (Duo-Spore®.) Inoculate into liquid culture as positive control for growth in liquid media and streak onto plates for positive control of agar media. Vegetative cells of *Bacillus stearothermophilus* do not survive well at <45 C. Therefore, controls should be maintained either as spores or at 55°C.

Negative controls. Incubate an uninoculated flask of medium with water samples at 55°C. Incubate uninoculated plates at 55°C with cultures. Culture and identify all contaminants.

Media storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Liquid media is also stable at 4°C for 30 days. Plates should be allowed to equilibrate to 55°C just prior to use.

Interpretation of results. Growth in liquid culture of inoculated water samples represents an enrichment culture and does not provide enumeration. All colonies should be identified and saved for determination of temperature profiles at a later date.

Confirmation. All colony types will be identified and saved for temperature profiles at a later date.

REFERENCES:

1) Duo-Spore® Data Card.

2) Gerhardt, P. 1981. Manual of Methods for General Bacteriology. American Society for Microbiology, Washington, DC, pp. 113-115.

ENUMERATION OF SULFATE REDUCING BACTERIA (CORROSION)

The sulfate reducing bacteria contribute to scale buildup and corrosion in water systems and to taste and odor problems. They are strict anaerobes that use organic compounds as a carbon and energy source and sulfate as a terminal electron acceptor. The sulfide produced by their metabolic activity is converted to sulfuric acid by thiobacilli, which can contribute to corrosion. Although these organisms are strict anaerobes, they can survive in biofilms where aerobic and facultative organisms reduce the oxygen tension.

Preparation of SRM liquid medium. Suspend 3.5 grams sodium lactate, 1.0 grams beef extract, 2.0 grams peptone, 2.0 grams magnesium sulfate heptahydrate, 1.5 grams sodium sulfate, 0.5 grams K_2HPO_4, and 0.10 grams calcium chloride in 1 liter of distilled, deionized water.

Ferrous ammonium sulfate solution. Suspend 3.92 grams ferrous ammonium sulfate $6H_2O$ in 100 mL distilled, deionized water and sterilized by **filtration**. Prepare fresh at the time of media inoculation.

Sodium ascorbate solution. Suspend 1.0 gram sodium ascorbate in 100 mL distilled/deionized water and sterilized by **filtration**. Prepare fresh at the time of media inoculation.

Preparation of Sulfate-reducing Medium Agar. Suspend 40 grams trypticase soy agar, 5 grams Bacto Agar, 4 grams sodium lactate, 2 grams magnesium sulfate, and 2.0 grams ferrous ammonium sulfate. Adjust pH to 7.3. These plates should be stored in a Gaspak within a hour after hardening to prevent saturation with oxygen. Petri dish covers should be replaced when necessary to prevent accumulation of moisture condensate.

Sterilization. Autoclave liquid medium at 15 lb pressure for 30 minutes (121^{o}C).

Quality Control Inoculate liquid media and agar medium with a stock culture of Desulfovibrio. Darkening of the medium is a positive control. Maintain uninoculated liquid tubes and agar plates as a negative control.

Storage. Sulfate-reducing medium may be stored at 4^{o}C for 30 days. Ferrous ammonium sulfate solution and Ascorbate solution must be prepared fresh daily. Sulfate-reducing medium agar must be stored in a Gaspak at 4^{o}C and used within 30 days.

ENUMERATION OF SULFATE-REDUCING BACTERIA (CORROSION) (cont)

Processing dirty samples. Serially dilute the water sample into the liquid growth medium (SRM) (6 dilution tubes) and then fill the tubes to the top with medium and attach screw cap. Add 0.10 mL ferrous ammonium sulfate solution and 0.10 mL Sodium ascorbate solution per 10 mL of medium. Incubate at room temperature and observe after 5, 10, and 20 days. The presence of sulfate reducing bacteria is indicated by darkening of the medium due to reaction of the sulfide with ferrous ions in the medium. Determine the approximate level of sulfate reducing bacteria present from the number of dilution tubes that appear darkened by 20 days.

Streak the last tube in the serial dilution that darkens onto SRM agar and incubate in a Gaspak jar until colonies appear (7 days). Restreak colonies onto SRM agar and incubate both aerobically and anaerobically to confirm.

Processing clean samples. Filter 10-100 mL of the water sample onto a 0.45µ filter and place the filter in a test tube containing SRM medium. Fill the tube to the top and attach screw cap. Add 0.10 mL ferrous ammonium sulfate solution and 0.10 mL sodium ascorbate solution per 10 mL of medium. Incubate at room temperature and observe after 5, 10, and 20 days. Presence of sulfate reducing bacteria is indicated by darkening of the medium due to reaction of the sulfide with errous ions in the medium. This method will not produce quantitative data, but will indicate the presence of sulfate reducing bacteria in clean water samples. If the liquid medium darkens, streak onto SRM agar and incubate in a Gaspak jar until colonies appear (7 days). Restreak colonies onto SRM agar and incubate both aerobically and anaerobically to confirm the presence of sulfate reducers. Record amount of water sample filtered.

Incubation. Incubate the cultures at 30°C and examined after 2, 10, and 20 days.

Interpretation of results. Presence of sulfate reducing bacteria is indicted by darkening of the medium due to reaction of ferrous with the sulfide produced. Colonies on the agar medium should be surrounded by a zone of blackening.

Confirmation. The last tube in the dilution sequence showing darkening should be streaked onto Sulfate-reducing Medium Agar and incubated at 30°C in a Gaspak.

REFERENCES:

Standard Methods for the Examination of Water and Wastewater 17th ed. 1989. American Public Health Association, Washington, DC pp. 9-114 to 9-126

Aaronson, S. 1970. Experimental Microbial Ecology. Academic Press, New York. pp. 116-119.

ENUMERATION OF POTENTIAL PATHOGENS (STREPTOCOCCUS)

Streptococci (e.g. Group A, hemolytic strep) cause a variety of diseases including pharyingitis, tonsillitis, impetigo, endocarditis and pneumonia. Transmission from person to person is associated with close contact with a colonized carrier. Many nonhemolytic streptococci (viridans) are part of the normal microbial flora of skin and mucous membranes. Some of these viridans streptococci have been implicated in endocarditis. Streptococci are fastidious and must therefore be grown on enriched media. Since they are aerotolerant anaerobes, they form small colonies on all culture media. Mitis-Salivarius agar supplemented with potassium tellurite is selective for viridans streptococci and enterococci, making possible their isolation from highly contaminated samples such as feces.

Preparation of media. Suspend 90 grams of Bacto Mitis Salivarius Agar in 1 liter of deionized/distilled water, using a 2-liter flask. Heat with continuous stirring allowing the medium to boil in order to completely dissolve the agar, then promptly remove the medium from the heat.

Sterilization. Autoclave at 121°C for 35 minutes. Cool medium to 50°C in a preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Addition of tellurite. Just prior to pouring plates, add 1.0 mL/liter of Bacto Chapman Tellurite solution. DO NOT HEAT THE MEDIUM AFTER THE ADDITION OF THE TELLURITE SOLUTION.

Dispensing. Aseptically dispense the sterile medium (7 ml) into sterile 50 x 9 mm Petri dishes with tight fitting lids and 100 x 15 mm standard petri dishes.

Processing dirty samples. Serially dilute (6 tubes) and spreadplate. After 48 hours, count colonies and transfer colonies to fresh plate to obtain pure cultures for identification.

Processing clean samples. Follow the Membrane Filtration Method described in the section on MEMBRANE FILTRATION. Aliquots of 100, 10, and 1 ml are to be filtered. Aseptically transfer the filters to plates. After 48 hours, count colonies and transfer colonies to fresh plate to obtain pure cultures for identification.

ENUMERATION OF POTENTIAL PATHOGENS (STREPTOCOCCI) (cont)

Quality Control. The dehydrated powder should be bluish beige and the prepared medium should be deep royal blue. Allow the agar to solidify and cool and then incubate at room temperature for 48 hours to permit adequate drying and as a check for contamination. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Controls. Streak a plate with *Streptococcus pyogenes* ATCC 19615. There should be good growth in 24 hours and the colonies should be blue.

Negative control. Streak a plate with *Escherichia coli* ATCC 25922 and another with *Staphylococcus aureus* ATCC 25923. There should be no growth after 24 hours.

Storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Incubation. Incubate aerobically at 37°C and examine after 48 hours. After two days, molds may appear. Count the number of colonies of each colony type and restreak a representative colony of each type for identification.

Interpretation of results. Enterococci for dark blue to black colonies. Any coliforms that are able to grow will form brown colonies.

Confirmation. All colony types will be identified.

REFERENCE:

Linette, E.H., A. Balows, W.J. Hausler and H.J. Shadomy. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington DC. pp. 154-160.

Difco Manual, 10th ed. 1984. pp.575-576.

ENUMERATION OF POTENTIAL PATHOGENS (STAPHYLOCOCCUS)

Staphylococcus is the most common causative agent of skin infections including boils, impetigo, wound infections, endocarditis, and pneumonia. This organism is also one of the most frequently isolated agents of nosocomial infections. Species most commonly associated with pathogenicity include S. aureus, S. epidermidis, S. saprophyuticus, S. intermedius, and S. hyicus. Normal flora staphylococci of low virulence include S. haemolyticus, S. hominis, S. warneri, S. saccharolyticus, S. cohnii, S. simulans, S. capitis, S. caprae, S. xylosus, and S. carnosus. This procedure is designed for the isolation and enumeration of staphylococci from water samples. The medium used contains 7.5% sodium chloride which is inhibitory to most bacteria.

Preparation of media. Suspend 111 grams of Bacto Mannitol Salt Agar in 1 liter of deionized/distilled water, using a 2-liter flask. Heat with continuous stirring allowing the medium to boil to completely dissolve the agar, then promptly remove the medium from the heat.

Sterilization. Autoclave at 121°C for 35 minutes. Cool medium to 50°C in preheated water bath. Do not allow the medium to stand for more than 1 hour between preparation and autoclaving.

Dispensing. Aseptically dispense the sterile medium (7 ml) into sterile 50 x 9 mm Petri dishes with tight fitting lids and 100 x 15 mm standard petri dishes.

Processing dirty samples. Serially dilute (6 tubes) and spreadplate.

Processing clean samples. Follow the Membrane Filtration Method described in the section on MEMBRANE FILTRATION. Aliquots of 100, 10, and 1 ml are to be filtered. Aseptically transfer the filters to plates.

Quality control. The dehydrated powder should be pinkish beige and the prepared medium should be red. Allow the agar to solidify and cool and then incubate at room temperature for 48 hours to permit adequate drying and as a check for contamination. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

Positive control. Streak a plate with Staphylococcus aureus ATCC 25923. There should be good growth and the medium should turn yellow within 24 hours. Also streak a plate with Staphylococcus epidermidis ATCC 14990. There should be good growth in 24 hours, but the medium should remain red.

ENUMERATION OF POTENTIAL PATHOGENS (STAPHYLOCOCCUS) (cont)

Negative control. Streak a plate with Escherichia coli ATCC 25922. There should be no growth after 24 hours.

Storage. The prepared plates may be stored at 4 C in sealed bags for up to 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Incubation. Incubate aerobically at 37 C and examine after 48 hours. Count the number of colonies of each colony type and restreak a representative colony of each type for identification.

Interpretation of results. Pathogenic staphylococci should form large colonies surrounded by a yellow zone. Nonpathogenic staphylococci should form small colonies with no color change to the medium. Nonstaphylococci should be inhibited, even when the medium is heavily inoculated.

Confirmation. All colony types will be identified.

REFERENCE:

Linette, E.H., A. Balows, W.J. Hausler and H.J. Shadomy. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, DC. pp. 143-145.

Difco Manual, 10th ed. 1984. pp. 559-560.

ENUMERATION OF SPORE FORMERS

Bacterial spores are highly resistant to temperatures that would destroy vegetative cells. The major genera of spore-forming bacteria are Bacillus (aerobes and facultative anaerobes) and Clostridium (strict anaerobes). Spores of various organisms differ in their heat resistance. Consequently, the length of time required to sterilize water depends on the kinds of spore-forming bacteria present and the levels of intact spores present. Levels of spores in water are best determined by pasteurization of the sample, followed by serial dilution and plating. Spores of Clostridium are not more heat resistant than those of Bacillus species. Therefore, enumeration of Bacillus spores should provide adequate information for design purposes.

Processing dirty samples. Place 5 mL of the water sample in a test tube and immerse in a water bath preheated at 80°C for 10 minutes. Serially dilute and plate onto trypticase (1:1) soy agar. Incubate plates at 30°C and examine after 48 hours. Enumerate colony types and restreak different colonies to obtain pure cultures. Identify each colony type and save for temperature profile to be determined at a later date.

Processing clean samples. Filter 50-100 mL of water sample onto a 0.45µ membrane. Transfer the entire filter to a test tube, add 5mL distilled, deionized water, and immerse in a water bath preheated at 80°C for 10 minutes. Serially dilute and plate onto trypticase (1:1) soy agar. Incubate plates at 30°C and examine after 48 hours. Enumerate colony types and restreak different colonies to obtain pure cultures. Identify each colony type and save for temperature profile to be determined at a later date.

Preparation of media. Suspend 15 grams of Difco trypticase soy broth and 15 grams Bacto agar in 1 liter of distilled, deionized water in a 2-liter flask and autoclave for 40 minutes. Allow media to cool to 50°C in a water bath and aseptically dispense into standard (100 x 15 mm) petri dishes. Incubate plates at room temperature for 48 hours before use to reduce excess moisture and check for contamination.

Note: This is one-half of the normal concentration of trypticase soy media. Experience has shown that this minimizes spreading of the colonies on plates. Spreading interferes with enumeration and isolation.

Quality control. The medium should appear light amber in color and translucent. A darkened medium may indicate an increased time or temperature in sterilization or charring during initial heating. Allow plates to incubate at room temperature, for 48 hours and inspect for contamination prior to usage. Record the number of contaminated plates in the Media Preparation Log Book. Discard all contaminated plates. If more than 10% of the plates are contaminated, discard the entire batch.

ENUMERATION OF SPORE FORMERS (cont)

Positive control. Prepare a spore culture of *Bacillus subtilis* as a positive control. The medium[1] consists of Difco Antibiotic Medium #3 (7.5 gm), Difco Peptone, (2.0 gm), Difco Beef Extract (3.0 gm), and 10 mL spore salts. Spore salts consists of $CaCl_2$-$2H_2O$, 13.0 gm; $MgCl_2$-$6H_2O$, 10.0 gm; $MnSO_4$, 2.8 gm; $ZnSO_4$, 0.5 gm, $FeSO_4$, 0.1 gm; $CuSO_4$, 0.1 gm; in 1 liter of 0.1 N HCL. Inoculate *B. subtilis* into 50 mL in a 250 mL flask and incubate with shaking (250 rpm) at 30°C. Maximum spore formation (about 10^6 spores/mL) occurs within a week of incubation. This culture may be stored at 4°C for several months without significant change in spore viability.

For use as a control, 5 mL of the spore culture should be processed as described above for a dirty sample.

Negative controls. Incubate a flask of medium inoculated with sterile water with water samples. Incubate uninoculated plates with cultures. Culture and identify all contaminants.

Media storage. The prepared plates may be stored at 4°C in sealed bags for up to 30 days. Liquid media is also stable at 4°C for 30 days. Plates should be allowed to equilibrate to room temperature just prior to use.

Interpretation of results. Enumeration of the various colony types from pasteurized samples will provide a count of the spores present in WRT water samples. Identification of the organisms will indicate heat resistance of spores. By combining data of kinds and numbers of spore formers, conditions for sterilization can be predicted.

Confirmation. All colony types will be identified and saved for temperature profiles at a later date.

REFERENCES:

1) Hungerer, K.D. and D.J. Tipper. 1969. Cell wall polymers of Bacillus sphaericus 9602. I. Structure of the vegetative cell wall. Biochemistry, 8:3577-3587.

2) Gerhardt, P. 1981. Manual of Methods for General Bacteriology. American Society for Microbiology, Washington, DC, p. 114.

APPROVAL

ANALYTICAL CONTROL TEST PLAN AND MICROBIOLOGICAL METHODS FOR THE WATER RECOVERY TEST

Revised by : M.S. Traweek and J.D. Tatara

The information in this document has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This document, in its entirety, has been determined to be unclassified.

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Qualitative and quantitative laboratory results are important to the decision-making process. In some cases, they may represent the only basis for deciding between two or more given options or processes. Therefore, it is essential that handling of laboratory samples and analytical operations employed are performed at a deliberate level of conscientious effort. Reporting erroneous results can lead to faulty interpretations and result in misinformed decisions.

This document provides analytical control specifications which will govern future test procedures related to all Water Recovery Test (WRT) Phase III activities to be conducted at the National Aeronautics and Space Administration/Marshall Space Flight Center (NASA/MSFC). This document addresses the process which will be used to verify analytical data generated throughout the test period, and to identify responsibilities of key personnel and participating laboratories, the chains of communication to be followed, and ensure that approved methodology and procedures are used during WRT activities. This document does not outline specifics, but provides a minimum guideline by which sampling protocols, analysis methodologies, test site operations, and laboratory operations should be developed.

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